ACTA OTO-LARYNGOLOGICA 76, 1–4, 1973 NARVAVÄGEN 16, 115 23 STOCKHOLM

SUBJECT INDEX

Acta Otolaryng 76: 1-10, 19—

ANATOMY AND PHYSIOLOGY OF THE O SET OF AUDITORY FUNCTION

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(Received September 5 1972)

Abstract Early cochlear microphonic and eighth nerve action potentials were recorded at th. round window in fetal and young guinea pigs, kittens and puppies. Their cochleas were later studied by light and electron microscopy Our results tend to confirm earlier studies about the main events and sequence of structural and functional development. Onset of function in the organ of Corti coincided with opening of the tunnel space, formation of the inner spiral sulcus and separation of the tectorial membrane. Correlation with electron microscopic findings shows that the earliest cochlear potentials follows by a few days early myelination and formation of both afferent and efferent neuro-epithelial synapses.

The earliest recordable round window re sponses were used in a number of previous studies to correlate structure and function of the developing auditory system. The opossum was studied by Larsell et al. (1935), the mouse by Alford & Ruben (1963), the rabbit by Ång gård (1965) and the cat by Pujol (1971). Friedmann & Bird (1959) introduced the use of electron microscopy for study of inner ear development in their work with isolated otocysts. Electron microscopy was used by Kikuchi & Hilding (1965) to examine mice at various stages after birth by Wersäll & Flock (1967) to study human fetal material guinea pig, mice and mink otocysts were studied by Nakai & Hilding (1968) and Hilding (1969) Sugiura & Hilding (1970) recorded phonic (CM) and action po-- the round window of the ga- later used for electron mic the cochleas. In that study (on the pathological findings r only a few observations were normal pattern of develop--

The purpose of this paper ; anatomical findings in var time of onset of auditory f by the appearance of coch.e to present a table of co-- ment.

MATERIAL AND M

Seven guinea pig and five ca tained by Caesarian delivery anesthesia of the mother estimated from the date of by reference to weight and 1920) Guinea pigs varied before birth (normal gesta 67 days), and cats from 6 to (normal gestation approx.- two cat litters, the Caesa performed aseptically to at different ages of the fe- 10 kittens and 15 puppies varying from birth to tw

Monopolar round w

This work was supported by NINDS Research Grant 03395 and by a grant from the Deafness Research Foundation.

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LIGHT MICROSCOPY

(Illustrations approx. × 350)
1 3 4 5 Toluidine blue.
2. Free section, Nomarski picture.



1

Hairs on hair cells. Large tectorial membrane attached to tall cells filling the internal spiral sulcus. Small fluid spaces in future tunnel. (Guinea pig -5 DBB)



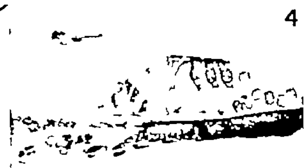
2

Tall cells of internal spiral sulcus have shortened to release part of the tectorial membrane. (Puppy 3 DAB)



3

Release of tectorial membrane and obvious tunnel space. Inner sulcus well formed. Stria vascularis has combined layers of epithelium and is vascularized. (Kitten 2 DBB)



4

Increase in tunnel and Nuel's spaces. Further atrophy of tall cells of inner sulcus. (Puppy 7 DAB)



5

Reticular plate tilted toward modiolus, pillars form. Hensen's cells developed. (Guinea pig DBB)

ELECTRON MICROSCOPY	ELECTRO PHYSIOLOGY	TIMING *(DBB, days before birth)* *(DAB, days after birth)*	
Early thickening of synaptic membrane. Outfolded cell wall of hair and supporting cells	No potentials	Guinea pig	30–24 DBB
Synaptic bars and cisterns appear in hair cells opposite nerve endings. Myelination of nerve fibres beginning	Possible to electrically stimulate auditory system, but no cochlear potentials	Guinea pig Cat Dog	20 DBB 5 DBB at birth
Probable increase in number of mature synapses	CM possible to elicit with intense stimuli	Guinea pig (Romand et al., 1971) Cat Dog Rabbit (Änggård, 1965) Rat (Wade, 1923 Crowley & Hepp-Raymond, 1966) Mouse (Alford & Ruben, 1963 Sher 1971)	15 DBB 2 DBB 4 DAB 4 5 DAB 8 DAB 11 DAB
Integration of stria vascularis epithelium	AP recordable at round window and entire auditory system responds to acoustic stimuli	Guinea pig (Romand et al., 1970) Cat (Romand et al., 1970) Dog Rabbit (Änggård, 1965) Mouse (Alford & Ruben, 1963) Mink (Sugiura & Hilding, 1970)	12 DBB at birth 7–8 DAB 5 DAB 1 –14 DAB 16 DAB
Hair cell mitochondria reduce in number and become arranged along the sides of the hair cells	Mature CM and AP with respect to threshold and frequency range	Guinea pig Cat Dog (approx.) Rabbit (Änggård, 1965) Rat (Crowley & Hepp-Raymond, 1966)	at birth 12 15 DAB 21 DAB [illegible]5 DAB 25 DAB

used to record CM and AP responses to loud click stimuli (around 90 dB SPL). The clicks were presented to the open bullae in free field The cochleas were promptly removed at the end of each experiment and prepared for light and electron microscopy Phosphate-buffered 3% glutaraldehyde was perfused through the perilymphatic space by opening round and oval windows. They remained in glutaraldehyde for 2 hours, were washed thoroughly in buffer and stained for 1 hour in 1% phosphate-buffered osmic acid After dehydration the specimens were imbedded in Epon The cochleas were divided and remounted on blanks before sectioning at 1–3 μm for toluidine blue light microscopy and 300–500 A for electron microscopy Thin sections were stained with uranyl acetate and lead citrate before being examined with an RCA 3G electron microscope

RESULT

Onset of auditory function

The earliest cochlear microphonic (CM) response was recorded in guinea pigs 15 to 12 days before birth (DBB) with intense click stimuli. In kittens, similar responses occurred 2 DBB and in puppies the first CM was recorded 4–5 DAB (days after birth)

Action potential responses (AP) were found a few days later In guinea pigs, the earliest AP was 12–8 DAB in kittens, birth to 2 DAB and in puppies 7–8 DAB In later stages, less intense stimuli were necessary and the frequency spectrum broadened (Pujol, 1971)

Structural maturation of the cochlea

The apical portion was less well-developed than the basal turn at every stage, as has been observed by many investigators previously and recording from the round window is a means of observing specially the basal part of Corti's organ Therefore our morphological studies concentrated on basal turn structures.

The time lag between the appearance of the two electrocochlear potentials led us to distinguish four stages during maturation of Corti's organ

1 Before onset of potentials. In our earliest specimens, guinea pigs 30 days before birth, hair cells had begun to differentiate stereocilia were present. The tectorial membrane had begun to form (Fig. 1) A short time before the onset of CM response (20 DBB guinea pigs 55 DBB kittens approximately at birth in the dog) the cochlea presented the following features: the hair cells were well but not completely differentiated. They were round shaped with a large nucleus. The tectorial membrane was completely formed but still strongly attached to the supporting and hair cells (Fig. 2). Neither the tunnel of Corti nor the Space of Nuel was visible. The internal spiral sulcus was filled with tall columnar cells but a small space had begun to form at the basal part of the cochlea.

2 Cochlear microphonic time· This stage occurred about 15 DBB in the guinea pig, ? DBB in the cat and 4 DAB in the dog The tectorial membrane detached from the supporting cells in the basal turn The tall cells of

Fig 6 Guinea pig inner hair cell (*ihc*) and tunnel region (*t*) from basal turn of a specimen estimated to be 23 days before birth. Although it was not obvious on light microscopy one can see that fluid spaces have already begun to form in the region of the tunnel. Outer hair cell (*ohc*). (All figures illustrate basal turn structures unless otherwise designated). ×4 000.

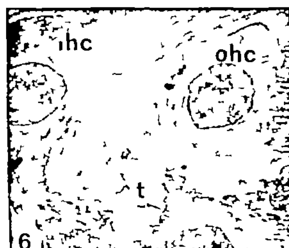
Fig 7 Kitten at same stage of maturation as Fig. 6, showing inner hair cell (*ihc*), outer hair cells (*ohc*), and tunnel area (*t*) with beginning formation of fluid spaces. Nerve fibers are seen crossing the fluid spaces (*arrows*) Age, 5 days before birth. 4 000

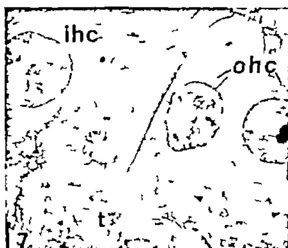
Figs 8 9 10 Nerve endings in guinea pig 3 days before birth, second turn.

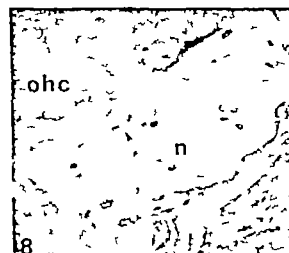
Fig 8 Nerve endings (*n*) beneath outer hair cell (*ohc*). Thickening was noted in small areas on both sides of the membranes. 11 000.

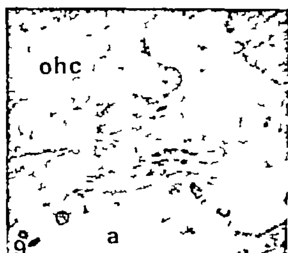
Fig 9 Slightly higher magnification of a presumably synaptic area between outer hair cell (*ohc*) and afferent ending (*a*) showing asymmetric membrane thickening. 16 500.

Fig 10 Early synapses between inner hair cell (*ihc*) and afferent nerve endings (*a*) with more pronounced asymmetry of membrane thickening (*arrows*). ×35 000


ihc
ohc
t
6


ihc
ohc
t
7


ohc
n
8


ohc
a
9


ihc
a
a
10

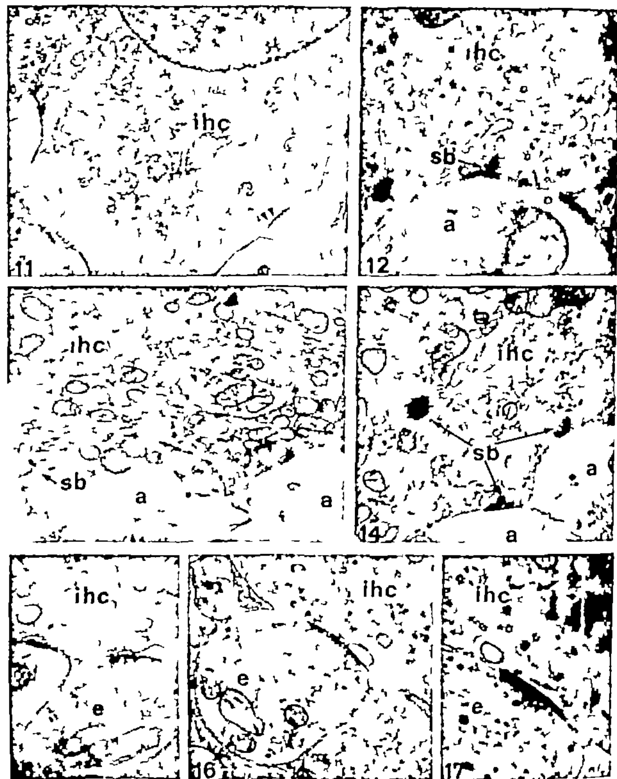


Fig 11 Inner hair cell of kitten 4 days before birth. Low magnification view showing cluster of afferent endings and an efferent terminal (*arrows*). Stage just prior to first CM potentials 6 000.

Fig 12 Same specimen, showing synaptic bar and membrane thickening. Inner hair cell. 45 000

Figs. 13 and 14 Puppies 3 days after birth at same stage physiologically as Figs. 11 and 12, just prior to CM Afferent endings were found beneath inner hair cells which contained synaptic bars (*sb*). 17 000.

Fig 15 Kitten 5 days before birth showing cistern in inner hair cell opposite efferent nerve ending. 20 000.

Figs. 16 and 17 Kitten 4 days before birth. Inner hair cell. Efferent endings (e) contain vesicles some with dense-core 23 000 (Fig. 16), 35 000 (Fig. 17).



Fig 18 Dog 3 days after birth showing early formation of myelin sheath around nerve fibers below the habenula perforata. ×36 000.

Fig 19 Kitten 4 days before birth, longitudinal section of myelinated nerve fiber in the lamina spiralis. 7 500

Fig 20 High magnification of the myelin sheath at the same age. ×36 000.

the "large swelling" had disappeared or become much shorter to form the internal spiral sulcus. The tunnel of Corti had begun to form (Fig. 3)

3 Action potential time: This stage followed the previous by 2 or 3 days. The outer hair cells had lengthened to approach their adult shape, with small nuclei near the lower end of the cells. The tunnel of Corti had well opened and Nuel's space had formed. Only a few tall cells remained near the inner hair cells in the internal spiral sulcus (Fig. 4).

4 Adult stage: As judged by light microscopy the adult form was reached 5 DBB in the guinea pig, 10 DAB in the cat and 15 DAB in the dog. It was characterized by the acquisition of the definitive hair cell form, a well-developed tunnel of Corti and tilting of the reticular plate towards the inner sulcus. The last group of cells to develop were the Hensen cells (Fig. 5).

Nerve endings and synaptic areas

Hair cells were well-differentiated, with cuticular plate and stereocilia in the earliest specimen we studied, the guinea pig 30 DBB. (Similar findings are illustrated in the second turn of a 23 DBB guinea pig and first turn of 5 DBB cat, Figs. 6 and 7) As seen in Figs. 8–10 the membranes are thickened in the synaptic area. In Figs. 9 and 10 the thickening is asymmetric and it is possible to conclude that these represent future afferent synaptic areas.

Two or three days before the CM could be recorded, efferent and afferent endings were identified. They were particularly evident near inner hair cells (Figs. 11–17) Like in the adult cochlea, afferent synapses were characterized by a thickening of the postsynaptic membrane and a synaptic bar on the presynaptic side surrounded by a cluster of vesicles (Figs 12–14) Efferent synapses were less numerous. As in the adult, they contained vesicles, some dense cored, and were found opposite a postsynaptic cistern in the hair cells (Figs. 15–17)

DISCUSSION

The purpose of our physiological recording was to correlate with histological findings. Our findings in guinea pigs agreed with previous experiments (Romand et al 1971) as did the results in postnatal cats with ... -ch-lear potentials (Romand et al

tory pathway activity (Pujol & Marty 1970 Pujol, 1971 and 1972). We found clear CM responses before birth as Romand (1971) tentatively reported. Zahlava (1966) and Fox (1968) recorded evoked responses from the dog's auditory cortex and stated that audition began about the 12th postnatal day Our method for recording showed somewhat earlier onset of function in dogs. However the dog still lagged about a week behind the cat in auditory development.

For many years, detachment of the tectorial membrane from its outer attachment has been regarded as an important morphologic finding that coincides with the onset of cochlear function The detachment is artifactual in life the tectorial membrane is attached to the organ of Corti (Hilding, 1952) However this attachment is so tenuous that routine histological techniques invariably show the adult tectorial membrane separated from the organ of Corti In the immature cochlea, the tectorial membrane is attached to the tall cells of the large swelling which fills the future internal spiral sulcus and extensively to the organ of Corti. The attachment is so broad that it would event relative movement and thus shearing ion or bending of stereocilia. At about the same CM was recorded, the large swelling had been replaced by the inner spiral sulcus releasing much of the early attachment of the tectorial membrane. Above the organ of Corti, the attachments became much less restricting. Lindeman et al. (1971) mentioned release of tectorial membrane outermost attachments as a function of maturation of the cochlea in cats. His observations at age one month are difficult to correlate with the much earlier onset and maturation of cochlear function

Two or three days after the CM was recorded, the earliest AP were found, confirming earlier observations in mice (Alford & Ruben, 1963) in rabbits (Änggård, 1965) and in guinea pigs and cats (Romand et al. 1970 and 1971) Lag of gross neural potential after CM of this interval is probably a general feature of mammalian development.

During maturation the earliest eighth nerve potential responses signal the beginning of function of the synapse between hair cells and afferent endings. We compared electrophysiological and electron microscopic findings on synaptic function and found that morphologic maturation (both afferent and efferent) preceded functional capability by about 2 or 3 days. The delay can not be ascribed to central pathway immaturity because the pathway is electrically excitable at an earlier stage (Marty & Thomas, 1963 Pujol & Marty 1968) Moreover an already forward process of myelination can be noticed around the lamina spiralis fibers, before appearance of potentials (Figs 18–20). Two explanations for this delay have been considered: First synaptic function depends on complex interactions involving transmitter substance inactivator special membrane characteristic and proper extracellular fluid. It would not be surprising if one or more of these essential factors appeared at a later time than the synapse attained morphological maturity Secondly the number of nerve endings may be insufficient and the quantitative aspect may be important as in other parts of the maturing nervous system (Aghajanian & Bloom, 1967) The AP represents the simultaneous discharge of a number of nerve fibers and a critical number of nerve endings may be required. Quantitative data would be needed to solve this question

Synaptic development in the organ of Corti is similar to the general pattern described for other portions of the nervous system by such authors as Glees & Sheppard (1964) Bunge et al. (1967) Woodward et al. (1971). The membrane thickens first, followed by an increase in the number of synaptic vesicles and mitochondria. In 1958, Engström clearly described the flat cisterns that are found opposite efferent endings in cochlear hair cells. The synaptic rod that is seen opposite afferent endings was depicted by Smith & Sjöstrand in 1961 The final stage of maturation of the neuro-epithelial auditory synapse is the appearance of synaptic rods and flat cisterns.

RÉSUMÉ

Les premiers potentiels microphoniques et potentiels d'action du VIII nerf ont été enregistré au niveau de la fenêtre ronde chez des foetus de cobaye et de chats, et chez des chiens et chats nouveau-nés. Leurs cochlées étaient ensuite prélèvées pour une étude en microscopie optique et électronique. Nos resultats tendent à confirmer de précédentes observations sur le développement anatomique et physiologique du système auditif. L entrée en jeu de la fonction auditive coincide avec l'allongement des cellules ciliées externes, l'ouverture du tunnel de Corti, la formation du sillon spiral interne et le détachement de la membrane tectoriale. Les corrélations avec les données *ultrastructurales* montrent que l'enregistrement des premiers potentiels est précédé de quelques jours par le début de la myélinisation des fibres nerveuses et par la formation des deux types de synapses, afférentes et efférentes.

ZUSAMMENFASSUNG

Frühe Aktionspotentiale der Cochlea und des achten Nervs wurden bei jungen Meerschweinchen, jungen Katzen und Hunden am runden Fenster mikrophonisch aufgenommen. Ihre Cochlea wurde später licht- und elektronenmikroskopisch studiert. Unsere Ergebnisse bestätigen frühere Resultate über Hauptvorgänge und die Abfolge der strukturellen und funktionellen Entwicklung. Die Funktion des Cortischen Organs setzte gleichzeitig mit der Öffnung des Tunnels sowie der Formung des inneren spiralförmigen Sulcus und der Absonderung der Tektorialmembran ein. Ein Vergleich mit den elektronenmikroskopischen Befunden zeigt, dass das früheste Potential der Cochlea einige Tage nach der frühen Myelination und Formung der afferenten und efferenten neuroepithelialen Synapsen erfolgt.

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Acta Otolaryng 76 11-16, 1973

TYPES OF NEURONAL ACTIVITY IN THE INFERIOR VESTIBULAR NUCLEUS

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(Received September 18, 1972)

Abstract Activity of 150 single units in the inferior vestibular nucleus was studied and neurons were classified according to their response. The response of neurons to a constant horizontal angular acceleration and deceleration of 4 /sec² revealed that 40.7% of those found did not respond to this stimulus. These were termed type V neurons and may represent projections onto the inferior vestibular nucleus mainly from the otolith organs. Type I (29.3%) neurons appear to be directly connected to the primary vestibular fibers innervating the horizontal semicircular ducts, and type II (27.3%) neurons are most likely projections onto the nucleus from the horizontal semicircular ducts via commissural pathway Only one type III neuron and three type IV neurons were found. The former are likely related to the efferent system and the latter to multisensory integration. The number of these two types is far fewer in this nucleus than the medial nucleus.

It has been already revealed that each vestibular nucleus is divided into zones whose cells receive different input from different sources (Brodal et al., 1962 Gacek, 1969 Stein & Carpenter 1967) and they differ in their properties and connections. In general, the vestibular nuclei receive input from the primary vestibular fibers, and also have fiber connections to and from the following regions in the central nervous system (CNS)· cerebellum, cerebrum, reticular formation, spinal cord and certain nuclei in the brain stem (Brodal & Høivik, 1964 Nyberg-Hansen, 1964 Scheibel & Scheibel, 1958 Trincker 1961 Yules et al., 1966)

This study was supported by Grant NS-06875 from the National Institute of Neurological Diseases and Stroke, National Institutes of Health.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The inferior vestibular nucleus (IVN also called the descending or spinal vestibular nucleus) is larger than the three other vestibular nuclei and most of its neurons are small to medium-sized. Anatomical studies show that the IVN receives fibers mainly from the otolith organs (utricle and saccule) and only its rostromedial parts receive some fibers from the semicircular ducts (Gacek, 1969 Stein & Carpenter 1967) Also fibers from the cerebellum supply the entire IVN (Brodal et al., 1962), and a relatively modest number of spinal afferents end here (Pompeiano & Brodal, 1957). In addition, its ventral portion receives fibers mainly from the contralateral IVN and some from the other three contralateral vestibular nuclei via commissural fibers (Ladpli & Brodal, 1968). The IVN receives fibers from other sources as well (Cajal, 1909· Torvik, 1956)

In previous studies the response of 128 single neurons in the medial vestibular nucleus (MVN) to caloric stimulation (Ryu et al., 1969) and 150 in the lateral vestibular nucleus (LVN) to rotatory stimulation (Ryu & McCabe, 1971) have revealed some knowledge of the neural activity in the vestibular nuclear complex. The experiments reported herein are concerned with the effect of rotatory stimulation of the

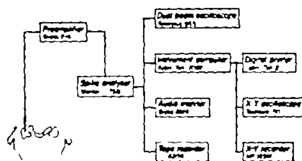


Fig 1 A block diagram showing the recording system.

horizontal semicircular ducts on the activity of single neurons in the IVN with special attention to the relationship between the morphology and physiology of the vestibular system

METHOD

Experiments were performed on 33 adult cats weighing 2.0 to 3.0 kg. Each animal was anesthetized with an intraperitoneal injection of 35 *mg/kg pentobarbital sodium*, and the cat was fixed in a stereotaxic apparatus. A tre phine was made over the posterior cranium, and the bony tentorium partially removed from each side An insulated tungsten microelec ode (impedance of 0.5 to 2.0 megohm) was ed stereotaxically in the area of IVN hrough the trephine and the cerebellum, using coordinates from the stereotaxic atlas (Ber man 1968 Snider & Niemer 1961) and Brodal's cytoarchitectural studies of the vestibular nuclei (Brodal et al., 1962).

The resting activity and perstimulatory ac tivity and responses were the parameters of interest and the results were analyzed with an on-line instrument computer (Fabri-tek 1062) for a frequency histogram of discharges. The action potentials (spikes) were displayed on an oscilloscope and recorded on an instrument tape recorder for data storage and for future analysis (Figs. 1 and 2) Audio monitoring was employed routinely The frequency histogram of spikes was displayed on the X Y oscilloscope and printed out on the Kodak linagraph paper using a high speed printer

When single neurons in the inferior vestibular nucleus were found resting activity was recorded (Fig. 3) for a period of 60 sec, and then the animal was subjected to a constant horizontal clockwise angular acceleration of 4 /sec² for 25 seconds, followed by an equal and opposite deceleration The initial and final velocities were 0°/sec with a peak velocity of 100 /sec at the end of acceleration phase. At the site of the last-studied neuron in each cat, a small electrolytic lesion was made and the brain stem was removed for serial sectioning and staining (Klüver & Barrera method) The locations of the neurons studied, but not marked electrolytically were determined by using the recorded coordinates of the locations of those neurons.

RESULT

Neural responses to a rotational stimulus of a total of 150 single neurons in the inferior vestibular nucleus were studied. All neurons recorded were tonic neurons (spontaneously active) and some variation in resting discharge rate was noted from unit to unit in the same preparation firing rate ranging from 1 0 spikes per second (sps) to 138 sps with overall average resting frequency of 23 sps. Neurons were classified according to their responses (Table I) to ampullopetal and ampullofugal flow of endolymph and this classification of neurons was used previously in the studies of medial and lateral vestibular nuclei (Ryu et al., 1969; Ryu & McCabe 1971).

Distribution of neurons according to type

44 (29.3) out of a total of 150 neurons were type I 41 (27.3%) were type II 1 (0.7) was type III 3 (2.0) were type IV and 61 (40.7%) were type V The average resting frequencies according to their type were 27 sps for type I 21 for type II 30 for type III 18 for type IV and 21 for type V (Table I).

Histological study showed that most of the neurons studied (129 neurons) were well in side of the IVN 15 neurons are near the

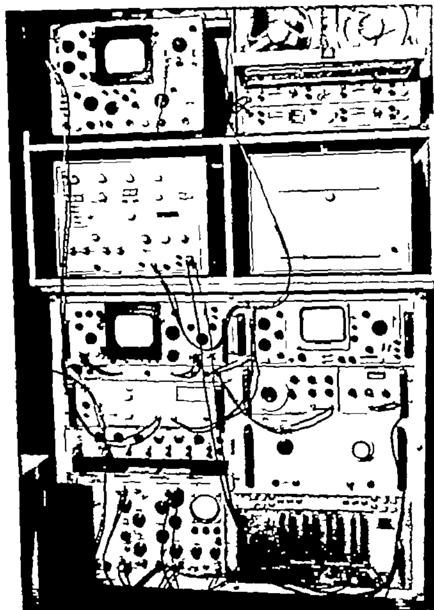


Fig 2 Display of recording and analyzing instruments.

boundaries of the IVN and the remaining 6 were outside the IVN boundary The six units that fell outside the usually accepted boundaries of the IVN responded to the given stimulus, hence were not excluded from the study

DISCUSSION

It appears that the main source of afferent fibers to the inferior vestibular nucleus is the vestibular end-organ (Gacek, 1969 Stein & Carpenter 1967). According to them, the rost romedial portion of the IVN receives fibers from the semicircular ducts, the dorsomedial portion receives fibers from the utricular ma cula, and the dorsolateral portion receives fibers mainly from the saccular macula. In addition, the caudalmost portion of the IVN receives a relatively modest number of spinal afferents (Pompeiano & Brodal, 1957) and fibers from the cerebellum (Brodal et al., 1. the medial portion of the IVN receives

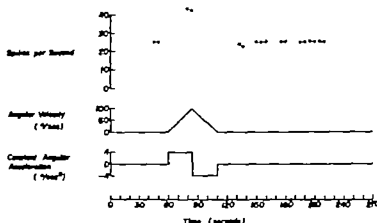


Fig 3 Profile of angular acceleratio and velocity used in the experimen and concurrent frequency of discharg of a type I unit in the inferior vestibul nucleus.

from the higher centers (Szentágothai & Rajkovitz, 1958) and the ventral portion receives commissural fibers mainly from the contra lateral IVN and some from the remaining three contralateral vestibular nuclei. The inferior vestibular nucleus also receives fibers from the reticular formation (Lorente de Nó 1933) a few fibers from the mandibular nerve (Torvik, 1956) the glossopharyngeal nerve (Cajal, 1909) and the cranialmost cervical dorsal roots (Corbin & Hinsey 1935 Yee & Corbin, 1939) Based on those anatomical findings, most of the single units in the IVN should not respond the horizontal semicircular duct stimulation and belong to the type V group (no change in neural activity to either direction of cupular deflection). Some neurons in the rostromedial portion of the nucleus should respond to rotatory stimulation. However we found a significant number of type I neurons (neuro showing increase in firing rate when the hor zontal cupula of the same side deviates t ampullopetal direction and decrease in firin rate to ampullofugal direction, Crampton' "Ewaldian unit") and type II neurons (neuro showing their response opposite to type I, Crampton's "non Ewaldian unit") and one type III neuron (decreased response to either direction of cupula deflection) and three type IV neurons (increased response to either direc tion of cupula deflection)

Type I and type II neurons are second-order neurons in the vestibular nuclei (Shimazu & Precht, 1966) which are connected with re ceptors in the semicircular ducts and activated by cupula deflection. Our 44 (29.3%) type I neurons in the IVN are most likely connected directly to the primary vestibular fibers inner

Table I *Classification and distribution of neurons in the inferior vestibular nucleus according to their responses to rotational stimulation*

Arrow up means an increase in frequency of action potentials occurred, arrow down, decrease N.C., no change

Types	Responses to rotatory stimulation: acceleration	Responses to rotatory stimulation: deceleration	No. of neurons	Percent	Average frequency (spikes/sec)
I	↓	↑	44	29.3	27
II	↑	↓	41	27.3	21
III	↓	↓	1	0.7	30
IV	↑		3	2.0	18
V	N.C.	N.C	61	40.7	21
Total			150	100.0	23

vating the horizontal semicircular ducts. Our 41 (27.3%) type II neurons are connected either directly to the fibers innervating the superior or the posterior ducts or more likely indirectly to the fibers of the horizontal duct via commissural fibers originating from the contralateral vestibular nuclear complex, since the vertical canals were stimulated minimally if at all in this experiment. Seventy-four of the 85 type I and II neurons responded to both clockwise and counterclockwise direction of rotation with specific polarity and remaining 11 neurons responded only to one direction of rotation.

There was only one type III neuron in the IVN. The number of type III neurons in the IVN is far fewer than in the medial vestibular nucleus. Type III neurons may be innervated directly from each end-organ without an intercalated inhibitory neuron or its response may be mediated through efferent vestibular system. The latter supports anatomic and neurophysiologic evidence that there are efferent fiber connections from the vestibular nuclei to the end-organ (Sala, 1965).

Type IV neurons in the vestibular nuclei exhibit an increase in activity after vestibular stimulation regardless of direction of rotation (i.e., no specific polarization). They respond to sensory stimuli such as pinching the ear or pulling a whisker. These neurons are thus non-vestibular in function, and probably connect to either pain receptors or the reticular formation. The latter supports the finding of Duensing & Schaefer (1957) who suggested that both type III and type IV neurons are functionally related to the reticular activating system. The number of type IV neurons in the IVN is much less than in the MVN.

The average resting frequency of single units in the inferior vestibular nucleus is 23 spikes per second which is the same as the average resting activity of MVN neurons but lower than that of LVN neurons (39 sps). One of the reasons for this low resting activity in the IVN is low resting activity of type V neurons (21 sps), which comprise over 40% of its population. Also, the resting activity of other types in the IVN is lower than the resting activity in the MVN and the LVN. If our type V neurons have a direct connection with the utricule and saccule, the resting discharge rate of the otolith organ is lower than the other vestibular receptors.

ZUSAMMENFASSUNG

Im unteren Vestibulariskern wurden die Aktivität von 150 Nervenzellen untersucht und die Neuronen entsprechend ihrer Reaktion klassifiziert. Aus der Reaktion der Neuronen auf konstante horizontale Winkelbeschleunigung und -verzögerung von 4°/sec² ergab sich, dass 40,7% dieser Neuronen auf diese Reizung nicht ansprachen. Diese Neuronen wurden als V-Neuronen bezeichnet; sie können in der Hauptsache Projektionen von den Otolithorganen zum unteren Vestibulariskern sein. Die I-Neuronen (29,3%) scheinen direkt mit den primären Vestibularisfasern, die die horizontalen Bogengänge durchziehen, verbunden zu sein. Die II-Neuronen (27,3%) sind höchstwahrscheinlich Projektionen zum Nukleus von den horizontalen Bogengängen über eine kommissurale Verbindung. Vom Typ III wurde nur ein Neuron und vom Typ IV wurden 3 Neuronen gefunden. Die ersteren stehen wahrscheinlich mit dem efferenten System in Verbindung und die letzteren mit der multisensorischen Integration. Die Zahl dieser beiden letzten Typen ist im unteren Vestibulariskern weit geringer als im medialen Kern.

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Acta Otolaryng 76 17–23 1973

FLUID SPACES IN THE SENSORY EPITHELIUM OF THE CRISTA AMPULLARIS IN GUINEA PIGS

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(Received September 18, 1972)

Abstract Intra-epithelial fluid spaces in the sensory epithelium of the crista ampullaris of guinea pigs were studied with the light and electronmicroscope. These fluid spaces were found in the intra-epithelial nerve axon just before giving rise to nerve chalices or even in the nerve chalice itself. Thus, these spaces appeared as large vacuoles in the intra-epithelial nerve axons. These axons were coming from thick myelinated nerve fibres. These spaces were usually from 1 to 5 in number and found exclusively in the central region of the crista ampullaris. Similar fluid spaces were sometimes detected in the nerve axons of thick myelinated nerve fibres before penetrating the basement membrane. The nature or the mode of origin of these intra-epithelial fluid spaces and the fluid spaces within the myelinated nerve axons was discussed.

The cochlear sensory epithelium—the organ of Corti—contains the tunnel space and Nuel s space which are said to be filled with cortilymph (Engström, 1960) which differs from the endolymph in its ionic and other compositions. On the other hand, the vestibular sensory epithelium has usually no special intra-epithelial spaces in its basic structure. Recently however Lindeman (1969) often found intra-epithelial spaces in the striolae of both maculae utriculi and sacculi and sometimes centrally in the cristae ampullares of guinea pigs in his systematic and quantitative study of the vestibular sensory epithelium. Lindeman suggested that these spaces are filled with a special fluid. The true nature of these spaces is, however still unknown

In the present study these intra-epithelial spaces were investigated in guinea pigs by means of the light and electronmicroscope with special reference to their relationship to the sensory cells and the nerve fibres.

MATERIAL AND METHOD

Five young (the exact age is unknown) non-albino guinea pigs weighing about 300 g were used in this study Within 3 minutes of decapitation the inner ear was fixed for electron microscopy by immersion in a 3% glutar aldehyde solution buffered at pH 7 4 with 0 1 M phosphate buffer for 1 hour at 0 C. Under the operation microscope, three ampullae were dissected out from the bony capsule in the above buffer solution. Afterwards the tissue was fixed with 2% osmium tetroxide (buffered with 0.1 M phosphate buffer at pH 7 4) for 1 hour at 0 C. The tissue was then rapidly dehydrated in ethanol, embedded in Epon (Luft, 1961), and sectioned in three planes. The ultrathin sections were double stained with an aqueous solution of uranyl acetate followed by a lead nitrate solution *a.m* Reynolds (1963). They were studied with the electronmicroscope (Hitachi HS-8)

OBSERVATIONS

Light microscopy

In the sensory epithelia of the cristae ampullares of all examined animals, fluid spaces were detected exclusively in the epithelium

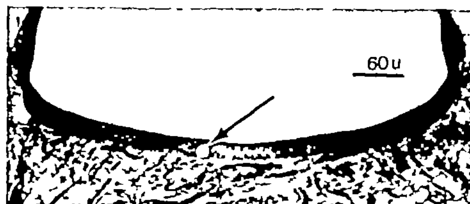


Fig 1 An intra-epithelial fluid space was detected in the central area of the sensory epithelium of the crista ampullaris (*arrow*). × 30.

of the central region of the crista ampullaris (Fig. 1) They were usually from 1 to 5 in number and from 10 to 25 μm in diameter varying in size and shape from one to another These spaces were found in the lower part of the sensory epithelium just above the basement membrane. These spaces were usually narrower basally and wider towards the surface of the epithelium. They never reached quite up to the surface of the epithelium Thick myelinated nerve fibres were found running up to the base of these intra-epithelial spaces. One of these nerve fibres, measuring about 10 μm in diameter contained a similar



Fig 2a.

Fig 2 (*a*) One sensory cell of type I was riding upon the space with the wide base of the cell (*S*). Penetration of the axon through the basement membrane was visible (*P*). 1 000. (*b*) Another sensory cell of type I (*S*) innervated by the chalice coming from the same axon. 1 000. (*c*) Penetration of the thick nerve fibre through the basement membrane losing its myelin sheaths and containing th space in the sensory epithelium These 3 figures were obtained from the successive series of 2 μm sections of the same fluid space. × 1 000.



Fig 2b.

long fluid space within the fibre before entering into the sensory epithelium (Fig 3)

Electron microscopy

Fluid spaces were all found in the nerve axon within the sensory epithelium. The lining walls of these intra-epithelial spaces were the nerve axons within the sensory epithelium after losing their myelin sheaths. The lining wall of one space so far as revealed in the present study consisted of one nerve axon which never encircled the space but contained the space in itself Thus, these spaces appeared as large vacuoles in the intra-epithelial nerve axons.

Peripheral to this space, the nerve axon gave rise to a few nerve chalices innervating bottle shaped type I (Wersäll, 1956) sensory cells (Fig. 2) Often 2-4 nerve chalices were found to derive from one intra-epithelial nerve axon. In these specimens, one or two chalices were found just above the fluid space but others were further away from it to some extent.

On the point of penetration of the nerve fibre, the axon containing the space was in direct contact with the basement membrane, losing its Schwann cell sheaths. In other places, however the nerve axon was found to be separated from the basement membrane by a few supporting cells. These supporting cells interposed between the basement membrane and the intra-epithelial axon constituting the basal wall of the space. On the basement membrane these supporting cells formed a rosette-like arrangement around the point of penetration of the axon through the basement membrane. The nuclei of these supporting cells were found in the cytoplasm beneath the space. It was not confirmed, however whether or not all these supporting cells reached up to the surface of the epithelium.

The axons and the nerve chalices contained



Fig 3 In the axon of the thick myelinated nerve fibre, a wide fluid space (*arrow*) was also found before entering into the epithelium. 400



Fig 2c



Fig 4 Fluid space in the nerve axon within the epithelium. The axon contained normal mitochondria, vesicles, filaments etc. × 2 400.

ny vesicles, mitochondria and some filaments (Fig. 4) The surface of the intra-epithelial axon facing the space consisted of one unit membrane which was usually smooth but sometimes finely irregular with occasional filamentous protrusions into the space.

In one specimen the intra-epithelial axon and the nerve chalice contained an amount of degenerated mitochondria, irregular dense debris, abnormal particles of moderately or well-organized lamellation figure varying in size and shape.

In the bottle-shaped sensory cells of type I embedded in this nerve chalice, a few mitochondria were found to be swollen with an interrupted internal membrane system and the cytoplasm appeared denser with the cell diminishing in size (Fig 5). The sensory cells of type I just above the fluid space were conspicuously shorter in height with the wide base of the cell riding upon the space. Sometimes other types of deformities of the basal part of the cell were also found in these sensory cells. The sensory cells of type I innervated by these nerve chalices, however usually showed no remarkable degenerative changes in the cytoplasm containing normal nuclei, mitochondria, endoplasmic reticula, ribosomes, vesicles, Golgi apparatus and sometimes round porous bodies of high electron density as are often found in normal sensory cells.

In two specimens of thick myelinated nerve fibres running up to the summit of the crista



Fig 5 An amount of degenerated mitochondria, irregular dense debris, abnormal inclusions of moderately or well-organized lamellation figure were found in the axon and in the nerve chalice. Sensory cell of type I (S) was reduced in volume and the cytoplasm appeared denser than in completely normal cells. × 2 400.



Fig. 6. Fluid space in the axon of a thick myelinated nerve fibre before entering into the sensory epithelium. One lamellation body (*L*) was found in the axon. The axon, however, showed no remarkable degenerative changes. The myelin sheaths were also well-configurated. Filamentous protrusions (*M*) of the axon into the space were seen. × 3 400.

ampullaris, similar fluid spaces were also found within the axon before entering through the basement membrane (Fig. 6). These spaces extended longitudinally in the axon, occupying more than three-fourths of the entire diameter of the lamellation ring of the myelin sheaths. These spaces ended just before the axon entered into the epithelium and no fluid space was detected within the axon in its course through the basement membrane. The axon contained many normal mitochondria, vesicles, neurofilaments and sometimes several lamellation bodies of high electron density. The surface of the axon facing the fluid space was rather smooth and consisted of one unit membrane. In the fluid space, a few filamentous protrusions coming from the axon were often found. The myelin sheaths of these nerve fibres were well-organized with no structural deformities.

DISCUSSION

The membranous labyrinth is small, enclosed in the hard bony wall of the temporal bone. Because of the complex structure of the labyrinth, especially its vestibular part, it is not always easy to make orientation in the operation. Furthermore, it is more difficult to irrigate the fixatives in the vestibular part than in the cochlear part. This means that the tissue of the vestibular labyrinth would often show post-mortem degenerative changes which must be excluded in a normal morphology. Thus, in the sensory epithelium of the vestibular apparatus, artefactual spaces would be produced due to poor fixation (Kolmer, 1927; Steurer, 1926).

In the present study, the spaces were found in the submicroscopically well-preserved sensory epithelium. In spite of the presence of spaces in the nerve chalice, for instance, the sensory cells embedded in it showed no remarkable post mortem degeneration. This also indicates that post mortem changes were excluded in this study.

Kolmer (1927) detected small intercellular spaces between the base of the sensory cell and the basement membrane due to poor fixation. The nature of these spaces, however, did not correspond to that found in this study. Werner (1933) first reported the presence of the cystic spaces in the well-preserved sensory epithelium of the macula utriculi in normal guinea pigs. He found these spaces exclusively in the striola of the macula utriculi. Neuman & Neubert (1958), however, found no such cystic spaces in the normal sensory epithelium of the macula utriculi at all. They detected these spaces only in the central region of the macula utriculi of guinea pigs treated with streptomycin compounds. These spaces were thought to be produced by swelling of the

basal part of the sensory cells in the process of degeneration.

Recently Lindeman (1969), however made a comprehensive study of the sensory regions of the vestibular apparatus and confirmed Werner's (1933) finding of these intra-epithelial spaces in the striola of a normal macula utriculi. According to Lindeman (1969), these spaces, varying in size and shape were wider towards the base, narrowing towards the apex and they did not reach the surface of the epithelium. The spaces were found less frequently in the striola of the macula sacculi and sometimes in the central part of the sensory epithelium of the crista ampullaris.

Lindeman (1969) presented an electron micrograph of one of these spaces in a horizontal section, showing the cytoplasmic lining around the space. It was not possible however to identify the nature of this surrounding cell.

In the present study these fluid spaces were found in the central region of the crista ampullaris in all the examined animals. They were identified as the spaces in the axon or in the nerve chalice of thick nerve fibres in the sensory epithelium after losing their myelin sheaths. Similar spaces were often observed in the axon of the same myelinated nerve fibres also before entering the epithelium.

It is not known whether these spaces are normal structures of a special function at all. Lindeman (1969) suggested that these spaces in the vestibular sensory area are filled with a special fluid different from both endolymph and perilymph in its composition.

A more probable concept is that they are produced by degeneration of the nerve fibres or of the sensory cells. If these spaces are large vacuoles exclusively confined within the nerve axons so far as confirmed in the present study it is more conceivable that these spaces are the result of degenerative changes in the nerve axons (chalices)

In the present study one nerve chalice possessing the intra-epithelial space contained many degenerated mitochondria, irregular dense debris and abnormal particles of moderately or well-organized myelin figures. Iurato (1967) was of the opinion that these cell inclusions were degeneration products due to aging or senile alterations. In other specimens, however no such degenerative products were found in the nerve fibres or in the nerve chalices. A more definite and quantitative evaluation of these spaces is necessary in the specimens of the aged animals as compared with those of the young ones.

Wersäll (1956) found in guinea pigs thick nerve fibres of 6–9 μm in diameter running up to the top of the crista finally giving rise to 3–4 nerve chalices in the epithelium. And according to Lindeman (1969) and Watanuki & Meyer zum Gottesberge (1971), several sensory cells (usually 2–4 in number) of type I were often found to be encircled in one nerve chalice in the central region of the crista epithelium. If one of these sensory cells, which are known to be most fragile in kanamycin and streptomycin ototoxicosis (Watanuki & Meyer zum Gottesberge, 1971), falls in collapse after degenerative swelling of unknown etiology a collapse space might temporarily be found in the nerve chalice. In the present study however no cell debris was found in the fluid spaces within the intra-epithelial nerve axons. Furthermore this assumption cannot explain the nature or mode of origin of the fluid spaces in the nerve axon before entering into the sensory epithelium.

ZUSAMMENFASSUNG

Es wurden die Flüssigkeitsräume im Sinnesepithel der Crista ampullaris bei Meerschweinchen licht- und elektronenmikroskopisch untersucht. Diese Flüssigkeitsräume fanden sich im epithelialen Nervenaxon oder im Zellkörper selbst. Sie erschienen wie grosse Vakuolen in den Neuriten, von denen dicke, myelinisierte Nervenfasern ausgehen. Diese Räume jeweils 1–5 an der Zahl wurden nur in der Zentralgegend der Crista ampullaris gefunden. Ab und zu fanden sich ähnliche Flüssigkeitsräume im Axon der myelinisierten, dicken Nervenfasern unter der Basalmembran.

Es wurde die Natur oder die Entstehung dieser

intraepithelialen Flüssigkeitsraume und dieser im myelinisierten, dicken Nervenaxon gefundenen Flüssigkeitsraume diskutiert.

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Acta Otolaryng 76 24–31 1973

INTERACTION OF OPTOKINETIC AND VESTIBULAR STIMULI IN MOTION PERCEPTION

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(Received July 28, 1972)

Abstract. The sensation of self-rotation (circular vection) was produced by rotation of a stripe pattern to the left or to the right at constant angular velocity. During circularvection, subjects were randomly accelerated in constant acceleration steps. The major experimental findings are:

1. Thresholds for detection of angular acceleration are raised when this acceleration is opposite to the direction of circularvection. Times to detect these accelerations are similarly increased.

2. Magnitude estimates of angular velocity show the effect of a visually induced velocity offset which is increased slightly by vestibular responses in the same direction and decreased markedly when the vestibular responses are in the direction opposite to self-rotation.

3. Many of the effects of angular acceleration on perceived velocity are accurately predicted by the adaptation model of the vestibular system. However, an important nonlinear interaction exists whereby rapidly occurring conflicts between visual and vestibular sensation, especially those involving direction disparities, result in a precipitous decline in circular vection and temporary domination by the vestibular response.

The sensation of self motion can be produced not only by vestibular stimulation, but also by excitation of the visual sense and by acoustic and somatosensory stimuli as well. Among the extra vestibular cues, visual motion information is by far the most powerful. Large moving scenes therefore are used in motion pictures, novelty rides, and flight simulators to produce remarkably strong illusions of motion through space (Barrett & Thornton 1968). Motion illusions resulting from combinations of conflicting visual and vestibular stimuli in aviation and space are well known and can cause serious disorientation.

Detailed quantitative data on interaction of visual and vestibular inputs and the site of this interaction are still lacking. This paper examines quantitatively the perceptual effects of visually induced self rotation on angular velocity sensation via the non visual (especially vestibular) senses and, conversely, the interaction of actual body motion with the visually induced motion sensation.

Although the sensation of self rotation associated with visual stimuli has been observed for rotations about vertical (Fischer & Kornmüller 1930, Brandt et al., 1971) as well as horizontal axes (Dichgans et al., 1972) this paper restricts itself to discussion of experiments in which the visual and vestibular stimuli represented rotation about a vertical axis. The self rotation sensation is, in many ways, *indistinguishable from* a vestibular sensation which might result from true angular acceleration in the dark. Perhaps most remarkable is the "pseudo-Coriolis effect" described by Brandt et al. (1971) and by Dichgans &

This work was supported by NASA Grants [illegible]2 009-156 and [illegible]-009-701. J. Dichgans was supported by the Sloan Foundation and the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 70). Th. Brandt was supported by the Fraunhofer Gesellschaft.

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Brandt (1972). Rolling the head sideways to the shoulder during visually induced self-rotation about a vertical axis results in a sensation of pitching forward or back similar to that sensation caused by a head motion made during a prolonged constant angular velocity of the body about a vertical axis.

The extensive literature on other motion illusions associated with visual and vestibular stimuli is reviewed by Peters (1969) and by Howard & Templeton (1966).

Mathematical models which describe the transformation from angular acceleration to sensation of angular velocity have been developed and tested for a wide variety of cases especially in rotations about a vertical axis (Young & Oman 1969). These models are based on data obtained while excluding visual input. Some quantitative information also exists on the relationship between pure visual stimulation and sensation of rotation in terms of the gain of the system, time delays to onset of circularvection, and the duration of circular vection outlasting the visual stimulus (Brandt et al. 1973). Virtually no quantitative data or models, however exist for the interaction between circularvection and vestibular stimulation.

The purpose of the experiments described in this paper is to answer the following questions concerning simultaneous visual and vestibular rotation stimuli about a vertical axis: (1) Does circularvection alter the thresholds to vestibular stimulation? (2) Can a vestibular stimulus suppress circularvection? (3) Do the contributions of vestibular and visual stimuli add linearly?

METHOD

The experiments entailed the projection of moving striped patterns on the front and side windows of a modified airplane flight simulator which, in turn, was rotated through a series of constant acceleration steps. The subject, seated inside the trainer with no view of the external surround, was required to make continuous magnitude estimations of his own angular velocity about the vertical axis. He also indicated his perceived direction of acceleration. The moving base simulator was modified from a standard Link GAT 1 flight trainer to permit independent control of angular motion about all three axes under the command of a hybrid computer. The simulator is capable of unrestricted displacement about the vertical axis at velocities up to 36.5 deg/sec with no noticeable discontinuity when passing through zero velocity. The subject was seated in the trainer and used a comfortable headrest which maintained the head in a normal upright orientation.

The visual stimulus was generated by a modified Kodak 600 slide projector equipped with an endless loop 35 mm film strip driven by a d.c. servo motor. A pattern of vertical black and white stripes, each subtending 138 arc minutes, was projected on the front and side windows of the trainer and translated horizontally. The horizontal visual angle subtended by each side window was 52 degrees and that by the front window was 84 degrees. The windows covered 52 degrees of visual angle vertically. The stripe velocity was either zero or 11.5 deg/sec.

A large illuminated dial placed 31 degrees below the horizontal was utilized by the subjects to indicate magnitude estimation of angular velocity. It was equipped with metal posts to permit its use in the dark. The depressed angle of this dial permitted the moving pattern to be entirely viewed peripherally. It had a range of ± 20 arbitrary units and was calibrated by each subject at the beginning of the experiment. The dial indicator was operated by the subject's right hand. With his left hand, he used a three position switch to indicate the perceived onset of angular acceleration to the left or right. Every effort was taken to eliminate any extraneous cues from the visual sources or sounds. Each experiment was run entirely under computer control.

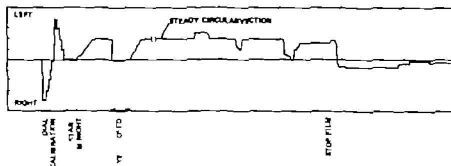


Fig. 1. Typical recording of magnitude estimation showing dial calibration, onset of circularvection, waxing and waning of circularvection with trainer stationary, outlasting circularvection following stopping of film, and long lasting aftereffect in opposite direction.

EXPERIMENTAL PROCEDURE

Four experimental conditions were utilized, entailing different visual stimuli. These consisted of (1) stripes moving to the left at 11.5 deg/sec (SL), (2) stripes to the right at 11.5 deg/sec (SR), (3) stripes stationary (SS), and, (4) subject blindfolded (B). Five subjects participated in the experiment. All of them were used to making consistent magnitude estimations, and all had normal vestibular function. The order of presentation of the four experimental conditions was balanced to eliminate order effects.

Two measures were taken during the experiment, a continuous recording of velocity magnitude estimation, and an indication of the time and direction of sensed acceleration signaled by the three position switch. To minimize habituation, subjects never received more than one experimental condition (less than 45 min) per day. Subjects were instructed to avoid head motion, while fixating the illuminated dial. They were reminded that they might frequently feel that their apparent velocity had been changed without actually having noticed any acceleration.

At the outset of each experimental trial, the magnitude estimation was calibrated by starting the film and having the subject track his estimation of velocity with the dial. The subject used a reference point 0 for no motion and ± 10 for the steady state self rotation at the 11.5 deg/sec film speed. This last estimate was found to equal film speed by counting subjects' indications of 90 degree angle increments. Once steady self-rotation was obtained, and indicated as 10, the subject was told to return the dial to 0 and close his eyes. After waiting 20 sec, he was told to reopen his eyes and immediately begin to track his velocity and signal accelerations. At this point the program to generate test acceleration steps was started.

In order to converge on vestibular thresholds, a sequence of stimuli modified from Clark & Stewart's (1968) double staircase technique was used. Acceleration tests approached threshold by two staircases, one from below and the other from above. When a step was correctly identified, the subsequent trial from that staircase was presented at a lower level. Similarly, when a step was missed or incorrectly identified the following step from that staircase was incremented. To converge rapidly, increments of acceleration were half the size of the decrements for the staircase starting at high accelerations, and vice versa for the staircase starting at low accelerations (Murphy, 1972). Minimum acceleration increments were 0.075 deg/sec². Thresholds to the left and right were determined independently.

RESULT

Interactions in velocity perception

A typical record of the calibration and onset of circularvection without any trainer motion is shown in Fig. 1. The onset of circularvection usually takes place over a 5 to 10 sec period, increasing rapidly toward an asymptote. The sensation of self-rotation corresponds to the stimulus velocity and often results in the perception of the stripes being stationary in space. Fig. 1 also indicates the characteristic

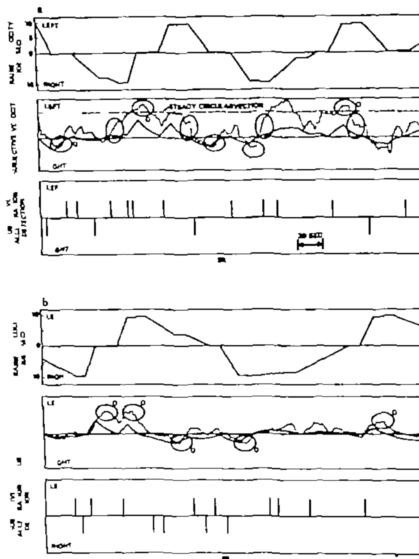


Fig. 2. Typical responses from one subject—two experimental conditions. (*a*) Stripes right. (*b*) Stripes stationary. *L*, Loss of CV. *G*, Gain of CV. *O*, Overshoot in CV following a period of constant acceleration.

waxing and waning of circularvection during constant velocity pattern motion. Following stopping of the film, with eyes open the circularvection lasts for an additional 3–5 sec, and decays quickly. It is followed by a prolonged aftereffect of sensation of slow self motion in the opposite direction to the original circularvection, lasting up to several minutes. During this last period the visual motion aftereffect usually is not perceived but may alternate with circularvection.

A set of representative records of subject II is shown in Fig. 2. The upper trace in each portion of the figure represents the angular velocity of the trainer. Maximum trainer velocity was 9 deg/sec. The second channel records the subject's continuous magnitude estimation of his angular velocity. The reference line represents his magnitude estimation of 10, corresponding to his calibration of self-rotation sensation at the stripe speed of 11.5 deg/sec. For the film moving to the right (2 *a*) the magnitude estimate is biased to the left, in the direction of circularvection. The heavy line on channel 2 is the output of a computer model which simulates the vestibular

Table I *Thresholds for detection of body angular acceleration in deg/sec, under various visual stimuli*
SL, stripes left; SR stripes right, SS, stripes stationary B, blindfolded. *t* calculated for difference in the mean threshold for

	Experimental condition									
	SL					SR				
	Acc. left		Acc. right			Acc. left		Acc. right		
Subject	Mean	σ	Mean	σ	*t* for means	Mean	σ	Mean	σ	*t* for means
I	0.67	0.24	0.22	0 09	4.30	0.60	0.19	0.85	0.12	2.78
II	0.33	0.08	0.28	0.10	1.04	0.10	0.08	0.31	0.10	3.96
III	0.19	0.10	0.08	0.04	2.56	0.21	0.06	0.32	0.09	2.39
IV	0.69	0.05	0.13	0.06	18.60	0.19	0.09	0.11	0 07	1 63
V	0.48	0.30	0.23	0.09	1.98	0.40	0.07	0.12	0.06	5 00

response to horizontal acceleration. This model, based on Young & Oman s (1969) adaptive vestibular model and using those nominal parameters, was subjected to the same input stimulus that the subject received.[1]

As can be observed from the film-still condition (2 *b*) many of the detailed features of the dynamics of the magnitude estimation are predicted by the vestibular model response However the effect of the moving visual stimulus is not merely to bias the response by the steady magnitude of self rotation but also to interact with the vestibular response. In particular at those points indicated on the records by L (for Loss) it is clear that a marked reduction in vestibular output or vestibular signal in the opposite direction to circularvection causes a precipitous loss in self-rotation The subjects sensation associated with loss of self-rotation were reported as a perception of a sudden onset of motion of the stripes, without any sensation of self deceleration. The duration and magnitude of trainer acceleration required to cancel circularvection is predictable on the basis of the calculated vestibular response A change in the direction of the vestibular output toward the direction of circularvection tends to promote an increase in circularvection. This is indicated by those places that are marked with a G (for Gain of self-rotation)

[1] The model can be represented by the following differential equation.

$$\int_0^t \omega(t)dt + 50\,\omega(t) + 600\frac{d\omega(t)}{dt} = \alpha(t) + 0.05\frac{d\alpha(t)}{dt}$$

where $\omega(t)$ is the sensed angular velocity and $\alpha(t)$ is the imposed angular acceleration.

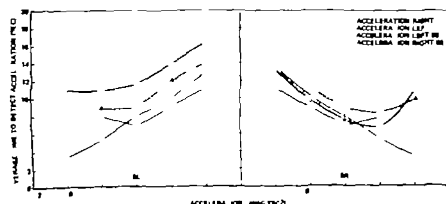


Fig 3 Average time to detection of acceleration steps, all subjects. Stripes still (*SS*) data are repeated on both sides of the graph Acceleration in the direction of stripe motion consistently yields large detection times.

accelerations left and right for each visual stimulus and subject. $n = 10$. $p < 0.05$ for $t > 1.8$. indicates difference not significant.

SS					B				
Acc. left		Acc. right			Acc. left		Acc. right		
Mean	σ	Mean	σ	t for means	Mean	σ	Mean	σ	t for means
0.40	0.10	0.32	0.06	2.55	0.45	0.07	0.41	0.11	0.78
0.13	0.08	0.22	0.04	2.50	0.35	0.17	[illegible]	0.07	1.46
0.17	0.09	0.17	0.13	0.00*	0.35	0.14	0.36	0.09	0.10
0.40	0.11	0.43	0.08	0.54	0.33	0.08	0.26	0.05	1.79
0.29	0.19	0.27	0.12	0.25	0.58	0.18	0.32	0.16	–.65

The observation that circularvection and vestibularly induced rotation do not add linearly can be quantified by a comparison of the vestibular model response with the magnitude estimates. Vestibular stimulation which tends to confirm or strengthen the circularvection contributes a modest addition to the magnitude estimation. On the other hand, a vestibular response opposite to circularvection seems to have a much greater result in decreasing the magnitude estimation, even in cases where circularvection is not completely destroyed. This observation is not an artifact of any magnitude estimation nonlinearity since magnitude estimation for circularvection has been established to be linear over a velocity range greater than that tested in these experiments (Brandt et al. 1973).

Visual Influence on "Vestibular Thresholds"

The effect of circularvection on sensation of vestibular inputs was examined both in terms of thresholds and the time for detection. As depicted on the third channel in Fig. 2, the subject's indication of acceleration lags stimulus onset by a varying duration. For the data analysis considered below therefore, the time delay from onset of an acceleration step to correct detection of this step was measured. Fig. 3 shows averaged results for all 5 subjects in which the time to detect is plotted versus the magnitude of acceleration step. Each data point represents the average time to detect the acceleration within a range of ±0.2 deg/sec². Cases of failure to detect the acceleration or indications of acceleration in the wrong direction were assigned an arbitrary time of 20 sec in calculating the averages. (Any detection of greater than 20 sec was considered a failure to detect.)

The accelerations to the right and to the left are separated for the SR and SL stripe motion conditions. The left and right acceleration curves for the SS condition are repeated on both halves of Fig. 3. Notice that the time to detect an acceleration in the direction of stripe motion (opposite to circularvection) tends to be 2–3 sec greater than for the same acceleration opposite to stripe motion. The curves for accelerations in the direction of circularvection closely match those for the stripe stationary and (not shown) the condition in which the subject is blindfolded.

Individual acceleration thresholds for the 5 subjects under the four experimental conditions are given in Table I. The thresholds were calculated by taking the mean of the lowest six correctly detected accelerations in each direction for each condition (accelerations which occurred prior to the eleventh step of the double staircase procedure were excluded in order to concentrate on the converged region).

Notice that in eight of the ten comparisons of SL and SR the mean thresholds were higher for accelerations in the direction of

tion than for those in the direction opposite to stripe rotation seven of these differences were statistically significant even with the small sample. That is, the effect of stripe rotation seems to relatively increase the acceleration thresholds for stimuli opposite to circularvection By way of comparison, seven of the ten cases for the B and SS conditions showed no significant left-right difference. The data suggest that with a stationary visual scene thresholds are lower than with the subject blindfolded.

Despite the different criteria used in this experiment than in many threshold experiments (our subjects were required to indicate sensation of acceleration by the switch and not merely the direction of velocity) the mean thresholds calculated here are close to those found in other threshold experiments reviewed by Clark (1967 1970)

DISCUSSION

Previous experiments have shown that visually induced self-rotation imitates a true vestibular sensation in many respects, including the quality of the sensation and the production ..pseudo-Coriolis effects. Electrophysiological ...ng from the vestibular nerve has shown ...) visually induced motion can produce ...als which can simulate the effect of an adequate stimulation of the semicircular canals (Klinke & Schmidt 1970; Dichgans & Brandt, 1972) The present experiments cast light on the way visually induced self-rotation and acceleration interact in the sensation of angular velocity about the vertical axis. The first major result is that thresholds for detection of angular acceleration are relatively increased when that acceleration is in the direction opposite to circularvection. Since cupula position is unaffected by visual input, this result is conclusive evidence that the "vestibular threshold" being determined by measurement of subjective sensation is not exclusively based on the mechanical properties of the cupula. The only established site of the interaction is through efferent synapse at the vestibular receptor (Klinke & Schmidt, 1970)

Visual-vestibular interactions seen in the magnitude estimation curves represent an interesting nonlinear interaction which would be difficult to explain solely on the basis of biasing a sensory cell through efferent stimulation. Angular accelerations which produce vestibular responses tending to confirm and increase the sense of self rotation associated with the visual stimulus are given a slight weight. On the other hand, acceleration patterns producing vestibular stimuli which are entirely contradictory to the visual stimulus, *in direction as well as magnitude frequently* destroy the self rotation sensation precipitously once they are noticed. Our current interpretation is that the conflict which can be tolerated between different sensory systems, supposedly monitoring the same body motion, is limited. When the conflict exceeds these limitations, a central process forces a decision as to which of the sensory signals is to predominate. In the current experiments, when the vestibular response is zero or in the direction of the circularvection the visual input dominates. However when the vestibular response suddenly indicates an angular velocity opposite to the visually induced sensation of self rotation the circularvection is destroyed or ignored without any sensation of sudden deceleration and the vestibular signal dominates for a time A schematic model of this mode of visual-vestibular interaction has been presented earlier (Young, 1970).

ZUSAMMENFASSUNG

Eigendrehempfindung (Cirkularvektion) wurde durch Projektion eines horizontal bewegten Streifenmusters ausgelöst. 5 Versuchspersonen wurden während der Cirkularvektion zusätzlich gleichförmigen, vestibulären Beschleunigungsreizen ausgesetzt. Die Ergebnisse waren folgende

1 Die Beschleunigungsschwellen für reale Eigendrehung sind höher wenn der wahrzunehmende Beschleunigungsreiz in Gegenrichtung zur Zirkularvektion gegeben wird. Auch die Erkennungszeit ist verlängert.

2 Geschwindigkeitsschätzungen zeigen, dass die

Zirkularvektion durch gleichgerichtete, vestibuläre Reize nur wenig zunimmt, während gegengerichtete Reize sie leicht aufheben.

3 Das vestibuläre Modell von Young und Oman lässt zahlreiche Effekte von Körperbeschleunigungen voraussagen, macht aber auch die Nichtlinearität optisch vestibulärer Interaktion deutlich.

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Acta Otolaryng 76 32–36, 1973

RELATION BETWEEN STRENGTH OF STIMULUS AND NYSTAGMUS FREQUENCY IN PATIENTS WITH MENIERE'S DISEASE

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(Received August 30, 1972)

Abstract Patients with unilateral Menière's disease were accelerated at 1 2° 4 and 8°/sec² Clockwise and counterclockwise accelerations were applied alternately Maximal nystagmus frequency was determined for each acceleration. The results were approximately the same on acceleration to the healthy and to the affected ear but at all acceleration strengths the patients with Menière's disease had a lower nystagmus frequency than the healthy subjects. This shows that the maximal nystagmus frequency cannot be used to demonstrate the differences between the right and the left vestibulooculur reflex in this type of stimulation. Nor can it be used to demonstrate vestibular recruitment. Nystagmus frequency is considered to be regulated by centra other than those regulating the latency time and the duration of poststimulatory nystagmus.

rotatory acceleration elicits a nystagmus ction that changes in many respects when ntensity of stimulus is increased. Thus, nystagmus starts after a shorter and shorter latency time (Fluur & Mendel, 1966), nystagmus develops its maximum intensity more rapidly (Fluur & Mendel, 1970) and poststimulatory nystagmus is successively prolonged (Fluur & Mendel, 1969) Patients with unilateral Menière's disease have been found to have nystagmus reactions which, in many respects, are distinctly different from those of healthy persons. Thus, patients with Menière's disease have vestibular recruitment as regards both latency time and duration of poststimulatory nystagmus (Mendel, 1971)

During recent years, nystagmus frequency has been regarded as a reliable indication of the vestibular function Torok (1969) investigated patients with Menière's disease by applying caloric stimuli of varying intensity and found that some of these patients displayed vestibular recruitment. In an earlier investigation of patients with Menière's disease we studied the latency time, crescendo time and after-discharge of nystagmus when different rotatory accelerations are used. The aim of the present investigation is to study how nystagmus frequency is changed when the intensity of stimulus is increased.

MATERIAL

A control group comprising 22 persons, mainly personnel of the Department of Otolaryngology were investigated. The Menière's material comprised 22 persons between the ages of 26 and 64 years, and the age distribution was the same as in the control group All these patients had a typical history of fits of dizziness, tinnitus and impairment of hearing on one side The impairment of hearing was on the left side in 14 patients and on the right side in 8 patients. Some subjects, however had slight impairment of hearing also in the other ear but this impairment was due to presbyacusis and affected the higher frequency range. Fowler's test was performed in each case to confirm that the impairment of hearing showed cochlear recruitment. All the subjects were also carefully examined by a neurologist in order to exclude the possibility of another disease in the CNS The investiga

tions were made during periods when the pa tients felt healthy had no dizziness, and took no pharmaceuticals.

Apparatus and mode of stimulation

The subject sat in an electrically-driven rota tion chair with the head secured in a holder and inclined 30 forward. The chair's axis of rotation was directed on a point half way between the subject s ears.

The experiment was preceded by a prelimi nary test The chair was accelerated at 1 /sec^2 for 20 sec and, after about 1 min at constant speed, was decelerated at 1 /sec^2 to a standstill. This preliminary test helped to familiarize the subjects with the procedure, and served as a final check of the rotation chair and the recording apparatus.

The actual experiment began with a clock wise acceleration at 1 /sec^2 for 40 sec fol lowed by at least 2 min at constant speed. The chair was then decelerated at the same intensity and brought to a standstill. After a pause of a few minutes, accelerations at 2 4 and 8 /sec^2 were similarly made for 40 sec, 30 sec and 20 sec respectively

Nystagmus was recorded with silver elec trodes placed at the outer canthi of each eye. The impulses were transmitted to a mingograph and the speed of the paper was 1 cm/ sec. The time constant was 2.5 sec and the amplification was such that 20 μV gave an amplitude of 10 mm.

The calculation was made when nystagmus had reached its maximal intensity and then remained constant. The first counted nystagmus beat was the one, following that slow phase, which formed the largest angle with the horizontal zero line (Fluur & Mendel, 1970). The nystagmus beats were then counted during 10 sec.

RESULT

Control material

A survey of the results shows that the differ ent individuals had wide variations in their nystagmus frequencies, but, there was throughout quite a regular increase in frequency when the acceleration strength was increased. At 1 /sec^2 acceleration clockwise the nystagmus frequency varied between 1 and 24 beats/10 sec, and the mean value was 9.5 at 2 /sec^2 the nystagmus frequency was between 3 and 25 beats/10 sec, and the mean value was 14.5 at 4 /sec^2 the frequencies were between 8 and 38 beats/10 sec, and the mean value was 19.6 Finally at 8 /sec^2 the frequencies were between 9 and 50 beats/10 sec, and the mean value was 25 6.

For counterclockwise acceleration the re sults were as follows: At 1 /sec^2 the nystag mus frequency varied between 5 and 19 beats/ 10 sec at 2 /sec^2 between 5 and 23 beats/ 10 sec at 4 /sec^2 between 9 and 34 beats/sec and, finally at 8 /sec^2 the nystagmus fre quency varied between 13 and 45 beats/10 sec. The corresponding mean values were 11.5 15 7 19.2 and 25 7 beats/10 sec respectively

On increasing the acceleration strength from 1 /sec^2 to 8 /sec^2 clockwise, the mean nystagmus frequency increased by 16.0 beats/ 10 sec. The corresponding increase was 14.2 beats/10 sec for counterclockwise accelera tion.

Patients with Menière s disease

About the same nystagmus frequencies were obtained when the accelerations were directed to the healthy as to the affected ear Thus, if acceleration was directed to the healthy ear the mean frequency at 1 /sec^2 was 8.1 beats/ 10 sec; at 2 /sec^2 11.8 beats/10 sec; at 4 /sec^2 15.5 beats/10 sec, and at 8 /sec^2 24.2 beats/ 10 sec (Fig. 1).

If on the contrary acceleration was directed to the affected ear the following frequencies were obtained at 1 /sec^2 8.6 beats/10 sec at 2 /sec^2 12.6 beats/10 sec; at 4 /sec^2 16.1 beats/10 sec and at 8 /sec^2 22.8 beats/10 sec.

In 13 cases out of the 19 acceleration to the affected ear showed a successive increase in nystagmus frequency on increasing acceleration from 1 /sec^2 to 8 /sec^2 whereas in 6 cases nystagmus frequency remained un

Table I. *Maximal nystagmus frequency (beats/10 sec) on stimulation at 1° 2° 4° and 8°/sec²*

Case no.	1°/sec²	2°/sec²	4°/sec²	8°/sec²	Case no	1°/sec²	2°/sec²	4°/sec²	8°/sec²
Normals									
Clockwise					*Counterclockwise*				
1	9	11	9	24	1	9	16	15	24
2	4	8	15	19	2	8	10	14	21
3	5	16	25	25	3	10	21	24	26
4	9	14	14	17	4	8	9	14	18
5	16	21	23	29	5	12	19	30	32
6	24	25	16	29	6	14	14	[illegible]3	30
7	4	21	33	33	7	10	19	16	25
8	11	7	8	12	8	9	5	10	13
9	8	19	21	22	9	13	23	18	28
10	12	10	11	9	10	16	13	20	24
11	12	18	38	50	11	12	22	26	45
12	1	11	22	31	12	16	9	15	20
13	11	3	17	26	13	15	18	16	[illegible]4
14	12	19	23	34	14	19	16	26	26
15	10	23	16	34	15	13	21	22	30
16	6	12	20	16	16	9	20	13	21
17	10	12	18	21	17	8	17	15	25
18	8	12	21	26	18	13	10	[illegible]2	29
19	14	14	21	19	19	9	13	20	23
20	4	17	18	23	20	9	18	9	19
21	9	6	10	24	21	5	15	20	27
22		20	33	39	22	15	18	34	36
$\bar{X}$	9.5	14.5	19.6	25.5	$\bar{X}$	11.5	15.7	19.2	25.7
S.D	5.0	5.9	7.8	9.2	S.D	3.4	4.9	6.3	6.7
C	0.53	0.40	0.40	0.36	*C*	0.30	0.31	0.33	0.26
Mb Menière[a]									
Acc. to affected ear					*Acc. to healthy ear*				
1	10	12	19	27	1	8	12	19	22
2	0	14	18	21	2	9	18	19	29
	11	11	19	20	3	6	4	15	26
	3	6	9	13	4	10	9	13	12
5	0	8	10	9	5	6	8	15	25
6	12	16	16	4	6	15	16	22	26
7	4	8	9	34	7	11	12	9	37
8	16	13	26	35	8	13	17	20	37
9	1[illegible]	16	17	26	9	8	16	19	23
10	12	13	21	27	10	10	14	21	28
11	16	20	17	20	11	0	7	8	25
12	2	7	12	24	12	4	10	13	17
13	13	9	4	19	13	11	11	7	18
14	16	21	28	29	14	12	16	22	31
15	8	13	11	17	15	8	12	19	24
16	8	11	16	19	16	11	13	14	18
17	9	13	14	19	17	4	7	11	17
18	11	18	20	27	18	8	12	14	22
19	0	10	19	23	19	0	11	15	22
$\bar{X}$	8.6	12.6	16.1	22.8	$\bar{X}$	8.1	11.8	15.5	24.2
S.D	5.5	4.2	6.0	6.5	S.D	4.0	3.8	4.7	6.5
C	0.64	0.34	0.37	0.29	*C*	0.50	0.32	0.30	0.27

[a] Menière's disease on the right side: Cases 1–8. Menière's disease on the left side: Cases 9–22.

changed or decreased when acceleration was increased from 1–2, 2–4 or 4–8°/sec². On acceleration to the healthy ear there was a successive increase in the nystagmus frequency in 14 of the 19 cases investigated, and in 5 cases the frequency either remained unchanged or decreased when acceleration was increased from 1–2, 2–4 or 4–8°/sec².

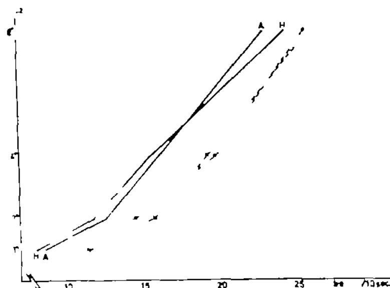


Fig. 1. Maximal nystagmus frequency in 19 patients with Menière's disease on acceleration to the affected ear (*A–A*) and to the healthy ear (*H–H*). The figure shows that the nystagmus frequency is approximately the same on acceleration to the affected and to the healthy ear and that it becomes increasingly higher when the stimulation intensity is increased. For comparison the maximal nystagmus frequencies of 22 healthy persons are also shown (O—O clockwise acceleration, ×—× counterclockwise acceleration). It is evident that the healthy persons had somewhat higher nystagmus frequencies throughout the entire acceleration range.

The total increase in the nystagmus frequency when increasing the acceleration from 1°/sec² to 8°/sec² was also calculated. This showed that the average increase was 16.1 beats/10 sec on acceleration to the healthy ear and 14.2 beats/10 sec to the affected ear.

DISCUSSION

In this investigation nystagmus frequency was observed to increase with increasing intensity of stimulus. In the control material the nystagmus frequency was somewhat lower for clockwise acceleration at 1° and 2°/sec² whereas at 4° and 8°/sec² there was no difference in the nystagmus frequency between clockwise and counterclockwise acceleration. These differences between vestibular reactions to clockwise and counterclockwise acceleration are in good agreement with earlier investigations, where it was shown that it is more difficult to elicit nystagmus clockwise than counterclockwise (Fluur & Mendel, 1967). A comparison with the patients with Menière's disease shows that they have a lower nystagmus frequency for the accelerations to both the affected and the healthy ear. This holds good for all the accelerations applied. Thus, the patients with Menière's disease tend to have somewhat depressed vestibular reactions for all the accelerations applied, but calculations have shown, that these differences are not significant.

By increasing the acceleration nystagmus frequency does not change in the same manner as the latency time and the after-discharge. Thus, nystagmus frequency shows no sign of recruitment.

The investigation also demonstrated that nystagmus frequency did not show any lateral differences in vestibular sensitivity such as were evident in the latency time. Possibly this is because the nystagmus frequencies were studied when the vestibular imbalance was very great, and that a much smaller vestibular imbalance is needed to enable detection of lateral differences. The latency period is measured at the beginning of stimulation, when this is less intense, and distinct lateral differences can then be observed. These findings are in good agreement with the results obtained by Torok (1969) when he applied strong and weak caloric stimulation to patients with Menière's disease. When nystagmus diminishes after the termination of stimulation, the vestibular imbalance is initially very great, and consequently lateral differences are not ob-

served when studying the after-discharge On the other hand, recruitment is observed. That maximal nystagmus frequency displays neither lateral differences nor signs of recruitment indicates that it is regulated by centres other than those controlling the latency time and the after-discharge.

ZUSAMMENFASSUNG

Patienten mit einseitiger Menièrescher Krankheit wurden abwechselnd im und gegen den Uhrzeigersinn mit 1 2 4 und 8 °/sec² beschleunigt. Bei jeder Beschleunigung wurde die maximale Nystagmusfrequenz festgestellt. Die Ergebnisse waren ungefähr gleich bei Beschleunigung hin zur Seite des kranken wie auch des gesunden Ohres. Bei allen Beschleunigungen hatten die Patienten mit Morbus Menière eine niedrigere Nystagmusfrequenz als die gesunden Vergleichspersonen. Die Untersuchung hat somit gezeigt, dass die maximale Nystagmusfrequenz nicht dazu verwendet werden kann, um bei rotatorischer Beschleunigung Unterschiede zwischen dem rechts- und linksseitigen vestibulookulären Reflex nachzuweisen. Ebensowenig kann mit dieser Methode ein vestibuläres Rekrutiment nachgewiesen werden. Es scheint, als würde die Nystagmusfrequenz von anderen Zentren reguliert als denen, die Latenzzeit und Dauer des poststimulatorischen Nystagmus bestimmen.

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Acta Otolaryng 76 37–46, 1973

SULFATED ACID MUCOPOLYSACCHARIDES IN THE TECTORIAL MEMBRANE

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Abstract The sulfated acid mucopolysaccharides in the tectorial membrane of the guinea pig were detected by the electron microscopic histochemistry with ruthenium red staining, microphotospectrometric measurements after alcian blue staining, and electron probe microanalysis for S element. Combining with enzymatic analysis, the subdivision of the sulfated mucopolysaccharides was determined. Since they were digested by testicular hyaluronidase and chondroitinase-ABC, the acid mucopolysaccharides spread in the tectorial membrane should at least partly be chondroitin sulfate A and/or C. These findings were discussed as well as evaluation of the electron microprobe analyzer The sulfated" acid mucopolysaccharides in the tectorial membrane would have a polarizing effect between the positive potential in the endolymph and the negative potential in the organ of Corti.

The presence of acid mucopolysaccharide (AMPS) in the tectorial membrane has recently attracted great interest not only for its elastic role but also for its possible polarizing role between the positive (ca +80 mV) potential in the endolymph and the negative (ca −80 mV) potential in the organ of Corti (Naftalin, 1965 Saito 1967) This has also been suported by an electrophysiological demonstration of zero potential in the tectorial membrane (Lawrence, 1967)

A great effort has been made to identify the chemical compontion, especially AMPS of the tectorial membrane, since AMPS would be capable of polarizing the membrane. The results obtained in various histochemical studies, however were not in agreement.

Iurato (1960) indicated no existence of mucopolysaccharides in the tectorial membrane because of the lack of hexuronic acid, although they might be present in such small quantities as not to be detectable by the method employed. Naftalin et al. (1964) also indicated a minimum of mucoid substance in the tectorial membrane because of a mere trace positive of hexosamine. On the other hand, Lotz & Kuhl (1970) detected rather larger amounts of mucopolysaccharide indirectly by hexosamine determination by column chromatographic separation from the whole membranous labyrinth According to a recent study by Saito & Daly (1970), 0.1% per dry weight of AMPS was detected in the tectorial membrane directly by turbidimetric method.

However the existence of sulfated" AMPS in the tectorial membrane still remains to be proved.

This study was undertaken to demonstrate the existence of sulfated AMPS in the tectorial membrane and to determine the type of sulfated AMPS.

METHOD

Twenty guinea pigs with a normal Preyer reflex, weighing between 250 and 350 grams, were decapitated. The petrous bones were isolated immediately and the bulla opened wide with a curette. Then under a stereoscopic

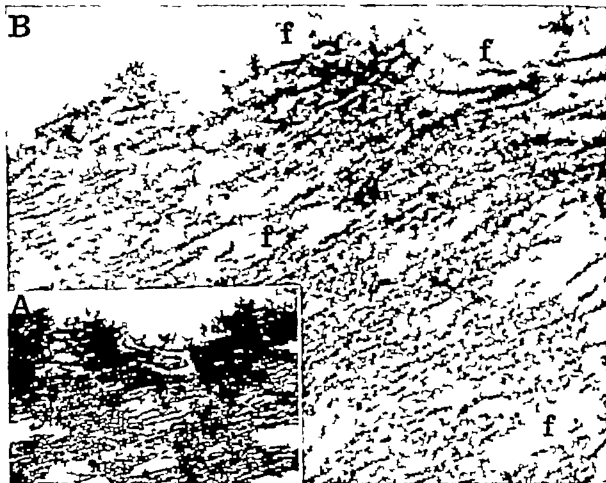


Fig 1 (A) Electronmicrograph of the tectorial mem- Ruthenium red staining. × 14 000 (B) A higher magnification view of (A). × 50 000 *f* fuzzy structure.

croscope, the dissections were made in the various manners described below

Electron microscopic histochemistry ruthenium red staining

The bony cochlea and stapes were removed and each turn of the cochlea was separated from modiolus in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7 4

The specimens were fixed in 1.2% glutaraldehyde with 500 ppm of ruthenium red in the buffer for 1 hour at 0 C. After washing in the buffer for 30 sec, the specimens were refixed in 2.0% osmium tetroxide with 500 ppm of ruthenium red in the buffer for 3 hours at room temperature.

Following dehydration in ethanol series, the specimens were embedded in Epon 812. Sections were cut for light microscopy and for electron microscopy at 300–600 A with glass knives on an LKB ultramicrotome and examined with an electron microscope (JEM 100B)

Digestive test

Testicular hyaluronidase (HAase) which decomposes chondroitin (Ch), chondroitin sulfate (ChS)-A, ChS-C and hyaluronic acid (HA) and chondroitinase (CHase)-ABC (Yamagata et al. 1968) which decomposes Ch, ChS-A, ChS-B ChS-C and HA were employed for this purpose

The procedure was as follows: The non-fixed

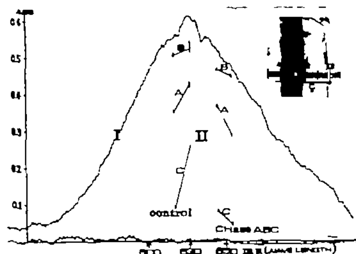


Fig. 2. (I) Absorption curve at a continuous wavelength at a certain point on the tectorial membrane stained with alcian blue. (II) Absorption at 630 nm at point A, B, C on the tectorial membrane (*right above*. Scale = 10 m) in two different visual fields. (*Right*) after CHase-ABC digestion (*left*) control.

cochlea, removed bony wall, and stapes, were delivered into four dishes.

The first dish (*a*) included 1 000 T.R.U of testicular HAase (Mochida Pharmaceutical Company Tokyo) in 10 ml of 0.2 M acetate buffer at pH 5.25 The second dish (*b*) was a control without HAase. The third dish (c) included 5 0 units of CHase ABC in 15 ml of 0 1 N Tris-HCl buffer at pH 8.0 with 50 mg of bovine serum albumin The fourth dish (*d*) was a control without CHase ABC. The dishes were incubated at 37 C for 12 hours. Then the tectorial membrane was peeled from the helicotrema to the basal end with fine forceps.

Microspectrophotometric measurements of AMPS after alcian blue staining

After the CHase-ABC digestive test the tectorial membranes were stained for AMPS by 1% alcian blue in 3% acetic acid for 5 min without any fixation spread over slide glasses and mounted in glycerol. Microspectrophotometric measurement was made with Shimadzu MPS-50 at a continuous wavelength from 500 to 800 nm. Then the transverse scanning measurement was carried out across the tectorial membrane at the maximal absorptive wavelength (630 nm) with a 50 × objective. The diameters of the scanning spot and the illuminated area were both fixed at 2 μm to eliminate Schwarzchild Villiger (S-V) effect. All the absorption curves in this procedure were recorded on the spectrophotometer

Electron probe microanalysis of sulfur (S) element

The tectorial membrane, with or without a digestive test, was fixed with 2.5% glutaralde hyde for 30 min and dehydrated in ethanol series. In this series, absolute ethanol without $CuSO_4$ was used to avoid the contamination of S element. The tissue was coated with aluminium and analysed for S element by the electron probe microanalyser (EMX SM Type Shimadzu, Tokyo). The electron beam was operated at the accelerating voltage of 20 kV with 0 14 ~ 0 46 nA sample current.

RESULT

Electron microscopic histochemistry

Filaments of indeterminate length were seen. These filaments had no periodic structure and had a diameter of about 150 A. They ran following a wavy course, mainly transversely and sometimes obliquely across the longitudinal axis. These filaments attained a high electron density and fuzzy structure arising from the

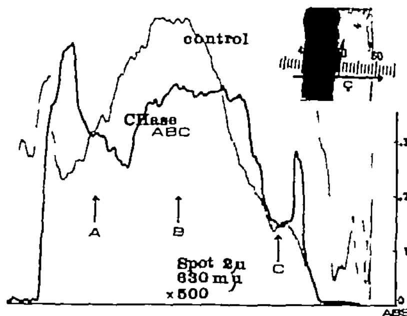


Fig 3 Scanning absorption curves at 630 nm along the transverse line ABC across the tectorial membrane digested with CHase ABC, and its control. ×325 Scanning spot and illuminated area both at 2 μm.

filaments became prominent after ruthenium red treatment (Fig. 1)

Microspectrophotometric study

The absorption curve at a continuous wave length at a certain point on the tectorial mem br stained with alcian blue showed the hape with a peak at 630 nm (Fig. 2) is absorption was thought to be "specific" alcian blue

In Fig. 3 both the scanning absorption curves along the transverse line ABC at 630 nm of the CHase-digested tectorial membrane and its control were seen. The mean absorption after digestion was higher than that of control in most cases. The absorption was high at the outer portion (*B*) and lower at the inner portion (*C*).

Study by EMX

The secondary electron image under high magnification showed clearly each part of the tectorial membrane (Fig. 4). The inner zone (*I*) inserted on the spiral limbus revealed a smooth surface and the middle zone (*M*) revealed many fibrils which ran practically parallel to each other and at an angle of about 40 to the transverse direction.

The outer zone (*O*) corresponding to the outer edge of the membrane, revealed a furrow-like structure

The X-ray image by SKα at the same site as in Fig. 4 brought out the diffusely distributed S element in the tectorial membrane (Fig. 5)

According to the line scan analysis across the membrane, the S element showed almost *the same concentration across the membrane,* but rather low concentration at the outer half of the middle zone The back-scattered electron (BSE) image and the X ray image by SKα after digestion with HAase were shown in Figs. 6 and 7 They revealed no evidence of the S element after HAase digestion compared with SKα of its control (Figs. 8 and 9)

The BSE image and the X ray image by SKα after digestion with CHase ABC and their control are shown in Figs. 10–13 They also revealed no evidence of the S element after CHase ABC digestion These images indicated that S element contained in the substance were decomposed away after HAase or CHase-ABC digestion



Fig 4 Secondary electron image of the tectorial membrane. *I* inner zone inserted on the spiral limbus; *M* middle zone *O* outer zone. 500.

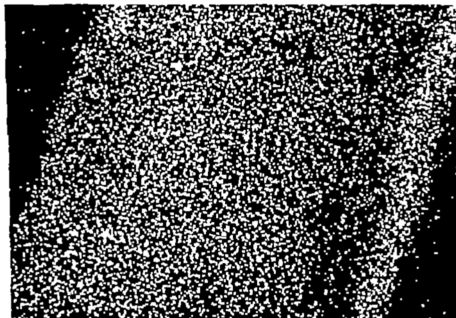


Fig 5 X-ray image by SKα at the same site in Fig. 4 500. S element spread diffusely



Fig 6 BSE image of the tectorial membrane after HAase digestion. ×100.

DISCUSSION

The electronmicroscopic histochemical study indicated that the tectorial membrane was consisted of non-collagenous filaments su rounded by ruthenium red positive AMP This structure supported the Dorfman s mod of ground substance (Rauch, 1964)

Fig 7 X-ray image by SKα at the same site in Fig. 6. ×100. S element was not detected.



Fig. 8 BSE image of the tectorial membrane. Control of HAase digestion test. 100

The microphotospectrometric study revealed that some of this AMPS might be HAase-digestive. However this method was not always accurate because of the different thickness of each tectorial membrane, distributional errors, proportionality errors, Schwarzchild-Villiger (S-V) effect, non-specific absorption and so on (Fukuda & Fujita, 1971).

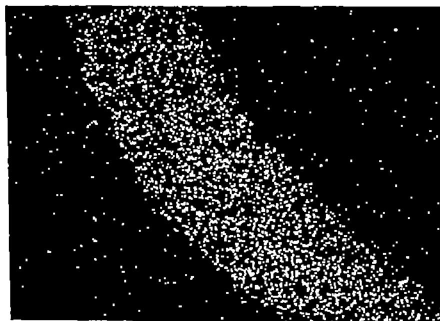


Fig. 9 X-ray image by SKα at the same site in Fig. 8. 100 S element spread diffusely

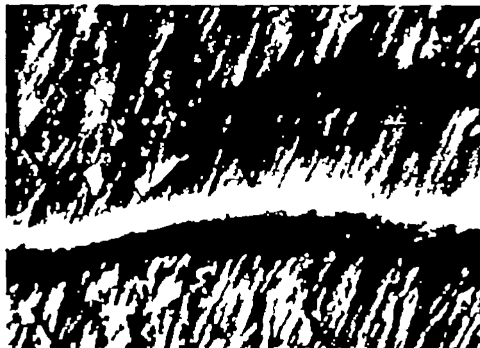


Fig 10 BSE Image of the tectorial membrane after CHase ABC digestion.

The EMX is a newly developed instrument. A beam of accelerated electrons travels from its filament to the specimen, exciting elements the specimens to emit X rays whose wave lengths are characteristic of the atomic number of that element. The intensity of the emission is proportional to the quantity of the excited element Any element with an atomic number

Fig 11 X-ray Image by SKα at the same site in Fig. 10. S element was not detected



Fig. 12. BSE image of the tectorial membrane. Control of CHase-ABC digestion test. ×100.

greater than 5 in concentrations as low as 10^{-15} g can be analysed. The EMX also has a spatial resolution of 1 μm as for X-ray image, allowing accurate tissue localization *of the elements which can be seen and photo*graphed (Doughman et al., 1970)

Combined with enzymatic analysis, the study with EMX revealed the existence of HAase

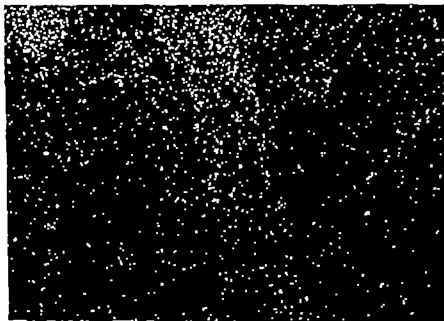


Fig. 13. X-ray image by SKα at the same site as Fig. 12. S element spread diffusely

and CHase ABC digestive sulfated AMPS which meant ChS-A and/or -C, spreading dif fusely on the tectorial membrane.

Iurato (1960) revealed 0 7 g of cysteine and cystine per 100 g protein in the tectorial membrane by the method of Mussini. Naftalin et al. (1964) showed by paper chromatographic separation, that there existed an S element in the form of sulphydryl and sulphide groups as in cysteine and cystine, but that there was no SO_4 in the tectorial membrane. On the other hand, the present study demonstrated an S element in AMPS in the tectorial membrane. After digestion, the S element was not detected in spite of the possible existence of cysteine and cystine. This result might be due to the small amount of cysteine and cystine compared with the sulfated AMPS, or due to the difference of their distribution. It was found in the present study that the tectorial membrane contained sulfated AMPS This sulfated AMPS would have a polarizing ef fect between the positive potential in the endolymph and the negative potential in the organ of Corti.

ACKNOWLEDGMENT

authors are grateful to Professor O Mizukoshi Dr M Fukuda for their suggestion and encouragement. The valuable technical assistance of Mr Katayama and Mr T Masaki is gratefully appreciated.

ZUSAMMENFASSUNG

Sulfomukopolysacchariden der Tektorialmembran von Meerschweinchen wurden durch folgende verschiedene, zytochemische und chemische Methoden untersucht: elektronenmikroskopische Histochemie mit Rutheniumrotfärbung, mikrophotometrische Messung nach Alcianblaufärbung, Sulfur-Analyse mittels Elektronenstrahlmikrosonde und Enzymanalyse Mit Hilfe der oben beschriebenen Methoden wurde nachgewiesen, dass die Tektorialmembran Chondroitinsulfat A und/oder C enthielt. Ihre mögliche Bedeutung für Struktur und Funktion des Hörorgans sowie die Bewertung der Elektronenstrahlmikrosonde wurden diskutiert.

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Acta Otolaryng 76 47–57 1973

THREE-DIMENSIONAL SURFACE REPRESENTATION OF THE CILIA FREE NASAL MUCOSA OF MAN

A Scanning Electron-Microscopical Study

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(Received July 3 1972)

Abstract The normal and the pathologically changed surface of the human nasal mucosa of the inferior conchae is described three-dimensionally with the aid of the scanning-electron-microscope Stereoscan. Scanning-electron-microscopical surface criteria are elaborated for differentiation of beaker cells, cilia-free cylindrical cells and squamous epithelial cells as well as for microvilli and re-proliferating kinocilliae The crossing effect of different-levelled cell borders and the appearance of different-levelled cell nuclei are typical of the squamous epithelium, they are not present in the cylindrical epithelium. The filled beaker cell shows a more homogeneous surface with small oval depressions and when compared with the surface of cilia-free cylindrical epithelial cells it presents a distinct decrease of microvilli-infestation. The microvilli show a ledge-like structure pattern whereas re-proliferating kinocilliae have papilliform, bud-like cytoplasmic bulges. In pathologically changed nasal mucosa, dome-shaped bulges and a marked loosening of the cilia-free cylindrical epithelium as well as a slow reduction of the microvilli structure are striking, while the squamous epithelium, in contrast to the physiological desquamation process, shows an intensified desquamation with widening of the intercellular spaces.

It is the aim of this investigation to describe the three-dimensional surface structures of the cilia-free nasal mucosa of man in its ultrastructure in order to find out whether pathologically changed ultrastructure can be differentiated from normal ones merely by looking at the surface with the aid of the scanning-electron-microscope (SEM)

Supported by the Deutsche Forschungsgemeinschaft. Lecture given at the V European Congress of Rhinology Barcelona, 22nd–24th June, 1972.

MATERIAL AND METHOD

The normal human nasal mucosa originates from the inferior nasal conchae and was obtained as described in another paper (Lenz, 1972). The pathologically changed nasal mucosa was obtained from patients with anamnestically detected vasomotor rhinitis by conchotomy under general anaesthesia and with extensive careful treatment of the surface. After operative removal, normal as well as pathologically changed mucosa strips are shaken in sterile physiological saline solution where they are kept for half an hour to several hours (Lenz, 1972) After thinning, the mucosa is spread onto a rigid plastic foil or cork and fixed with needles the surface is faced upwards. A mucosa prepared in this manner is fixed in 5% buffered glutaraldehyde solution for 1/2 to 4 hours. Subsequently the preparations are dehydrated in alcohol of ascending concentration (30 40 50, 60 70, 80, 90, 96, 100 and 100%) while the dehydration time in the corresponding alcohol is 1/4 hour each and the total dehydration time $2^1/_2$ hours. Before fixation, some of the preparations are kept in 5% buffered saccharose solu-

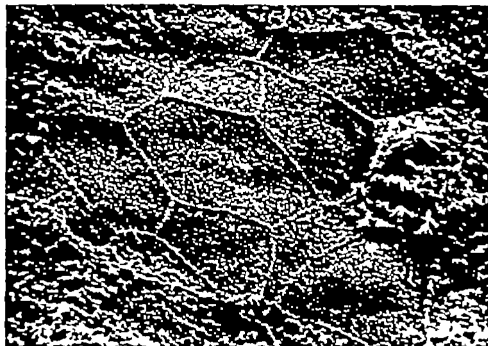


Fig. 1. Cilia-free cylindrical epithelium of normal nasal mucosa in man. (*a*) Closely joined cylindrical cells with polygonal ledge-like cell border. Hexagonal shape in the centre. Re-proliferation of [illegible] particularly on the right. SEM, × [illegible] 640.

tion for several days. Subsequent to the dehydration the mucosa strips are cut in pieces of up to 0.5 cm² and immediately placed on round metallic optical carriers. The prepara- are re-dried in high vacuum and coated ·h gold, the coating layer being 300 to 500 .. A total of 25 pathologically changed mucosa strips of the inferior conchae were obtained from 13 different patients. Forty-one scanning-electron-microscopical preparations were produced and examined from 10 persons, normal mucosa was obtained, from which 27 SEM preparations were produced and examined. The SEM examinations were performed with the Stereoscan (Cambridge Scientific Instruments).

RESULT

Cilia free cylindrical epithelium

The cylindrical epithelium of the normal nasal mucosa in the anterior third of the inferior nasal conchae displays cilia-covered cylindrical epithelial cells as well as cilia free ones (Jahnke, 1972. Lenz, 1972). Normally they show a closely knit epithelium formation with narrow ledge-like cytoplasmic elevations as cell borders (Fig. 1 *a*). They are almost identical in height and sharpness of outline. In contrast to squamous epithelial cells a crossing of cylindrical cell borders is not observed. The cell borders of cylindrical cells form polygonal areas of various size. Hexagons are seen (Fig. 1 *a*). The nuclei of the cylindrical epithelial cells cannot—in contrast to those of the squamous epithelial cells—be represented.

In some parts of the normal cilia-free cylindrical epithelium, openings of glandular ducts are seen. Most of them are partially filled with mucus. This mucus can be emptied granular or string-like (Fig. 1 *b*).

The ultrastructure of the normal cilia-free cylindrical cell shows a fine-dimensioned ledge-like pattern in the shape of narrow cytoplasmic elevations which are the equivalent of the microvilli of the cylindrical cell surface. Additionally smaller bud-like papillary cytoplasmic apical bulges of almost the same size



Fig. 1 b. On the right, glandular duct with string-like emptying mucus and polygonal apical ledge-like cylindrical cell border. SEM, ×2 400.

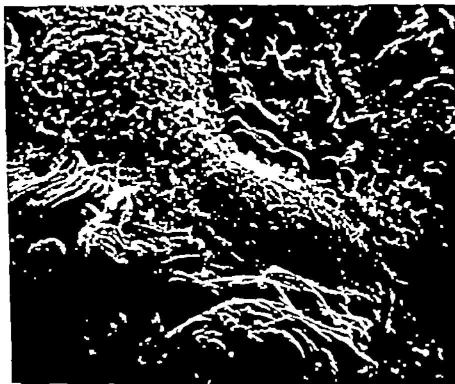


Fig. 1 c. Confrontation of different ultrastructures: Ledge-like microvilli-infestation in the centre, papilliform re-proliferations of kinocilia top left. Longer tube-like kinocilia top right and bottom left. SEM, ×6 000.

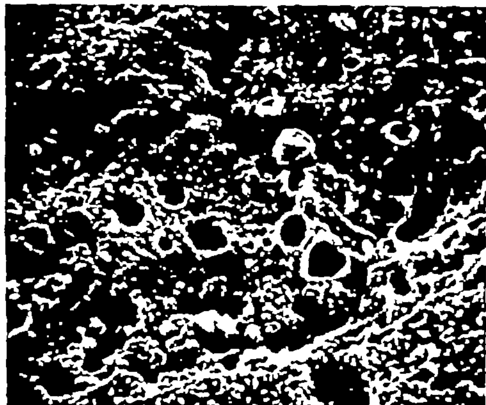


Fig 2 Beaker cell surface from human nasal mucosa, with reduced microvilli-infestation and oval depressions. SEM × 6 000.

are present. These are reproliferations of kinocilae (Dalen et al. 1971 Lenz, 1972) (Fig. 1 c). They are found on the surface of both normal mucosa and that in vasomotor rhinitis.

In the cilia-free cylindrical epithelium, beaker cells can be particularly well demonstrated under the scanning-electron-microscope Compared with the cilia-free cylindrical cell surface, the filled beaker cell shows certain typical ultrastructural surface characteristics (Fig. 2): oval depressions and pores of various size a more homogeneous surface structure and a distinct reduction of the microvilli infestation. The emptied beaker cells appear to be funnel-shaped or as oval depressions (Fig. 3) Larger depressions refer to adjacent empty beaker cells.

In vasomotor rhinitis, empty beaker cells with large mucous plugs on the epithelial surface can be seen next to beaker cells which are still filled. Here observation of the surface shows the disturbed dispersion from beaker cells to cylindrical cells particularly clearly (Fig 3).

Besides a preponderance of beaker cells, cilia-free cylindrical cells can be seen which show a distinct microvilli-infestation or re-proliferations of ciliae. In vasomotor rhinitis a more intense appearance of cilia free cylindrical epithelium is evident. Apart from closely joined cylindrical cells with visible cell borders, cilia-free cylindrical epithelial cells with dome-shaped bulges and no recognizable cell borders are present (Fig. 4 a) In vasomotor rhinitis mostly a distinct loosening of the cilia-free cylindrical epithelium is observed—the cells disintegrate and wide intercellular spaces become visible (Fig. 4 *b*). The widened intercellular space of the cilia free cylindrical epithelium can be observed particularly well in the SEM cross-section picture (Fig 4 *c*). Furthermore, a degeneration of the cylindrical epi-

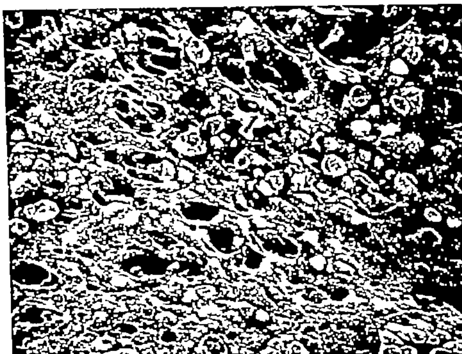


Fig. 3. Empty beaker cells of human nasal mucosa in vasomotor rhinitis. Preponderance of empty beaker cells as oval depressions and filled beaker cells with reduced surface differentiation. Dome-shaped cylindrical cells. Larger mucus plugs on the surface. SEM, × 600.

thelium is seen with plaque-like destruction of the cells and a slow decrease and loss of the microvilli-infestation. In the widened inter cellular spaces round cells are present. The dif fering ultrastructures of short kinociliae, ledge like microvilli and beaker cell surfaces are elaborated.

Squamous epithelium

By scanning-electron-microscopy the squamous epithelium of the normal mucosa is seen as a closely joined epithelial formation which, in some places, shows a physiological desquamation (Fig. 5 *a*). The cell borders appear as narrow ledge-like elevations and can be interpreted as cytoplasmic marginal tori of the epithelial cells (Fig. 6). The cell borders differ in outline and height. The brighter out lined, more superficial cell borders correspond to the epithelial cells close to the surface, the darker and lower ones to the more deeply located epithelial cells. Crossing of shallowly and deeply located cell borders is observed (crossing phenomenon of cell borders) thus forming polygons of various sizes (Fig. 6). Considering the outline intensity of the single cell borders, polygonally shaped epithelial cells of approximately the same size are found in the corresponding space. The surface of the single epithelial cell shows fine-dimensioned ledge-like cytoplasmic elevations similar to microvilli patterns (Fig. 5 *a*). In some places in the normal squamous epithelium oval depressions and pores of 300 to 400 Å are found between the microvilli (Fig. 5 *c*).

At the cell borders, more or less sharply outlined oval to round elevations at different levels are found to be cell nuclei (Fig. 5 *a*, *b*). The prominent cell nuclei correspond to epithelial cells lying near to the surface, and having sharply outlined cell borders, less sharply outlined cell nuclei can be regarded as more deeply located epithelial cells (Fig. 5 *a*, *b*).

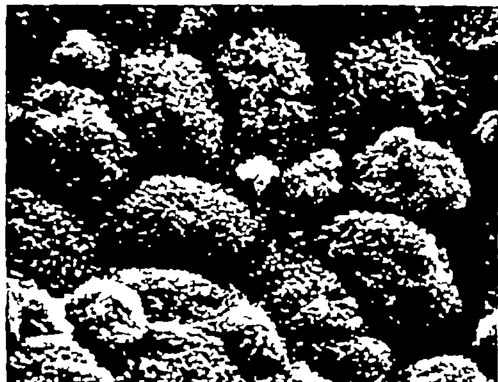


Fig 4 Cilia-free cylindrical epithelium of human nasal mucosa in vasomotor rhinitis. (a) Dome-shaped cilia-free cylindrical epithelial cells without visible cell borders. SEM, ×4 032.

Cell nuclei and cell borders shine through the cytoplasma of epithelial cells close to the surface. The extremely thin quality of the cytoplasma of a squamous epithelial cell becomes evident in its terraced localisation. The step-like character becomes obvious in the cytoplasma outlined in marginal tori and in the different microvilli patterns of the epithelial cells (Fig. 5 *b*) In some epithelial cell nuclei, on magnification a smaller elevation almost in the centre of the nucleus is interpreted as a nucleolus.

The physiological desquamation process of the squamous epithelium which has been referred to in another paper (4) is present only in some places of the normal mucosa. An intensified desquamation of the squamous epithelium (beyond the physiological rate) extends over larger mucosa areas and is observed during vasomotor rhinitis in the frontal zones of the inferior nasal conchae (Fig. 6). Besides squamous epithelial cells located side by side dispersed areas with disintegrated cell borders are seen In the areas with an intensified desquamation the intercellular cleft appears widened. The epithelial cells show a marginal partial lifting of their cytoplasma, bulges, pleatings and even a turning up of the cell margin areas. *Single* epithelial cells or bigger cell formations separate completely In the epithelium there remains a flat, not always visible, desquamation cell.

DISCUSSION

On comparing the surfaces of squamous- and cilia free cylindrical epithelial cells in the human nasal mucosa by use of the scanning-electron-microscope, the following criteria result concerning the differential diagnosis:

(1) While in the epithelial cell the cell nuclei are visible—due to the extremely thin quality of the cytoplasmic layer—this is not the case when using our SEM preparation method on

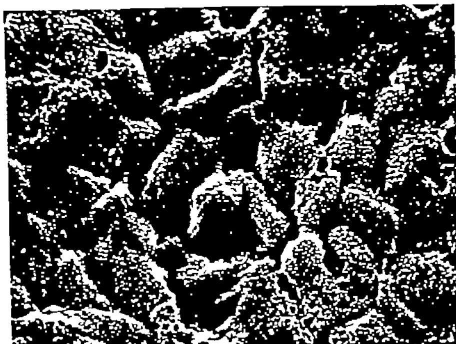


Fig 4 b Strongly loosened cylindrical epithelium with wide intercellular spaces. SEM, 750.

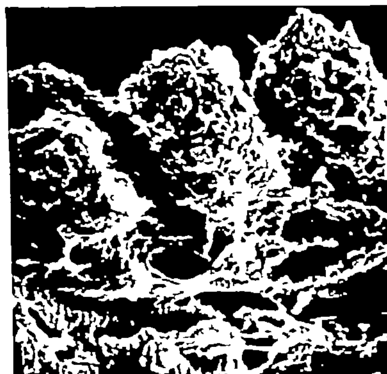


Fig 4 c Cross-section of a scanning-electron-micrograph. Three cylindrical cells on the basal membrane are seen at the bottom of the picture with a wide intercellular space, particularly in the centre of the picture. SEM, 5 000.

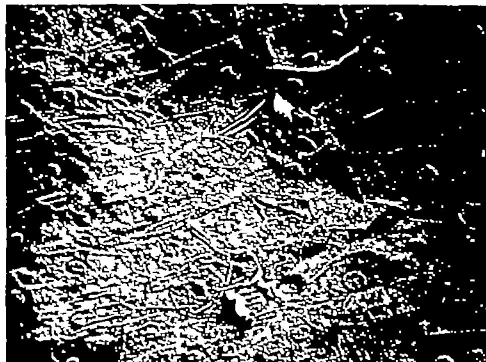


Fig 5 Normal squamous epithelium of human nasal mucosa with physiological desquamation. (*a*) Cell nuclei and cell borders. Sporadically rising epithelium and epithelial desquamation cells. SEM, ×6 000.

the cylindrical epithelial cell, since these nuclei are covered by a substantially thicker cytoplasmic layer

(2) The crossing effect of the squamous ..ial cell borders at different levels which can be observed with the scanning-electron-microscope is not present in the cilia-free cylindrical epithelial cells, since these are situated side by side and not on top of each other

(3) The terraced position evident in the different microvilli patterns and the cytoplasmic cell borders made prominent by their marginal tori, is characteristic of the thinness of squamous epithelial cells. It is not found in cylindrical cells.

(4) The flat epithelium depression is a typical condition after desquamation of the squamous epithelium. It is not present in the cilia free cylindrical epithelium.

The physiological desquamation process of the squamous epithelium of the normal mucosa can be easily differentiated from an intensified desquamation process in vasomotor rhinitis with separation of mainly larger epithelium formations. An intensified desquamation process such as shearing-off of larger epithelial areas—similar to the conditions in vasomotor rhinitis—has hitherto only been observed in animal experiments on the hamster cheek pouch immediately after cryosurgical treatment and is interpreted as a typical cryosurgical change which is found on thawing (Lenz, 1972)

We consider the oval depressions between the microvilli (size 300 to 400 A) which are found in some parts of the normal squamous epithelium to be cytoplasmic dells in the cell surface in order to take up larger foreign particles. As these depressions are not always present we think that they occur only in a certain functional state of the cell.

The microvilli of the cilia-free cylindrical epithelial cells are of a more ledge-like fine-dimensioned cytoplasmic pattern whereas re-proliferating ciliae show more papilliform

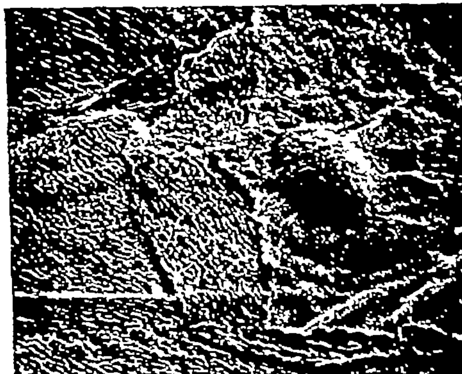


Fig. 5 b. Terraced position of squamous epithelial cells with different microplicae patterns and variously levelled cell borders and cell nuclei. SEM × 2 880.

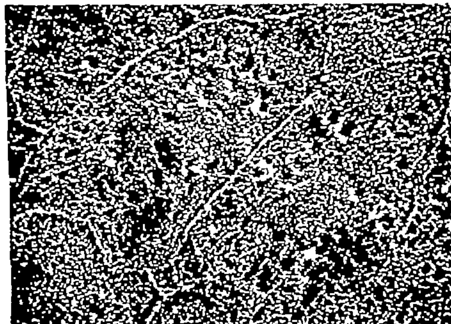
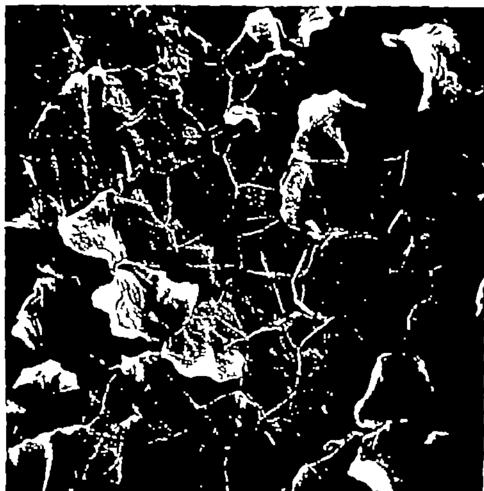


Fig. 5 c. Between the fine-dimensioned ledge-like microvilli, small oval depressions are seen similar to cytoplasmic depressions of squamous epithelial cell surface, expressing a certain state of function. SEM, 2 280.



6 Squamous epithelium of the human nasal ..sa in vasomotor rhinitis. Desquamation of larger epithelium complexes. Variously-levelled crossing cell borders. SEM, × 600.

bud like cytoplasmic bulges of approximately the same size Thus reproliferating ciliae can be differentiated from microvilli under the scanning-electron-microscope. The papilliform re-proliferations of kinociliae correspond to the kinociliae produced *in vitro* by other authors (Dalen et al. 1971)

By means of the scanning-electron-microscope the cilia free cylindrical cell can be differentiated from the filled beaker cell by its relatively homogeneous surface, oval pores, and a reduced microvilli infestation the infestation of the surface of cylindrical cells is particularly distinct.

The strongly loosened cylindrical epithelium in vasomotor rhinitis indicates an extraglandular transudation which has been observed electron-microscopically by other authors (Jahnke 1972 Terrahe & Backwinkel 1970)

ACKNOWLEDGMENT

The experiments were carried out in the Central Institute of Electron Microscopy Technical University of Berlin, with the aid of the scanning-electron-microscope Stereoscan, donated by the Stiftung Volkswagenwerk. I am greatly indebted to Professor Helmcke for supporting my studies, to Mr Neubauer and our Photographical Department for their technical assistance

ZUSAMMENFASSUNG

Die normale und pathologisch veränderte Oberfläche der menschlichen Nasenschleimhaut der unteren Muscheln wird dreidimensional mit Hilfe des Rasterelektronenmikroskops Stereoscan beschrieben. Die

rasterelektronenmikroskopischen Oberflächenkriterien zur Unterscheidung von Becher zilienfreien Zylinder und Plattenepithelzellen sowie von Mikrovilli und neusprossenden Kinozilien werden herausgearbeitet.

Der Oberkreuzungseffekt von niveauunterschiedlichen Zellgrenzen, die Darstellung von niveauunterschiedlichen Zellkernen sind typisch für das Plattenepithel und fehlen beim zilienfreien Zylinderepithel. Die gefüllte Becherzelle besitzt eine mehr homogene Oberfläche mit kleinen ovalären Vertiefungen und weist gegenüber der zilienfreien Zylinderzelloberfläche deutliche Reduzierung ihres Mikrovillibesatzes auf. Die Mikrovilli zeigen ein leistenförmiges Strukturmuster wogegen neusprossende Kinozilien papillenförmige knospenartige zytoplasmatische Vorwölbungen besitzen. Bei pathologisch veränderten Nasenschleimhautoberflächen sind kuppenförmige Vorwölbungen und eine starke Auflockerung des zilienfreien Zylinderepithels sowie eine Verdümmerung der Mikrovillistruktur auffällig, wobei das Plattenepithel gegenüber dem physiologischen Abschilferungsvorgang eine verstärkte Abschilferung mit Aufweitung der Interzellularräume zeigt.

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Acta Otolaryng 76 58–62, 1973

DOUBLE BLIND TRIAL OF DOXICYCLINE IN ACUTE MAXILLARY SINUSITIS

A Clinical and Bacteriological Study

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(Received September 20, 1972)

Abstract Sixty-one patients, 39 females and 22 males, with bacterial and sterile acute maxillary sinusitis were subjected to study in order to decide whether or not an oral antibiotic is necessary in the treatment of this condition when it is administered simultaneously with irrigation and nose drops. Using a double blind method, one group of patients were given doxicycline and one group a placebo. Changes in secretion were followed macroscopically and changes in the nasal patency rhinomanometrically Significant differences between the recovery time of patients belonging to the doxicycline and placebo groups were not observed, neither did the sensitivity of bacteria from the secretion to doxicycline correlate with the speed of recovery in either group. The results do not indicate that recovery time in acute maxillary sinusitis can be shortened with oral administration of doxicycline.

There are several treatment methods for acute maxillary sinusitis, but irrigation of the maxillary sinuses is the measure most commonly employed. Often, nose drops and an oral antibiotic (usually penicillin or a tetracycline) are added to the treatment. Nevertheless, many persons find irrigation unpleasant, and it has to be replaced by other treatment. In these cases antibiotics improve treatment results (Axelsson et al., 1970) As the micro-organism found in maxillary pus often is *Haemophilus influenzae* (Urdal & Berdal, 1949 Kortekangas, 1963 Lystad et al., 1964) drugs of the tetracycline group might be preferable. Many authors, however recommend penicillin as the primary antibiotic in the treatment of acute maxillary sinusitis (Sparrevohn & Buch, 1946 Axelsson et al., 1970)

With an oral dose of 200 mg of doxicycline a peak serum concentration of 2.59 μg/ml is reached within 2 hours 15 min (Fabre et al., 1966). After 24 hours, the concentration is still 1 45 μg/ml. If from the second day on, the dose is 100 mg, then the serum concentration remains at 1.5–3 0 μg/ml (Fabre et al., 1968). Using a daily dose of 200 mg a concentration of 0.5–7.5 μg/ml is obtained in maxillary pus (Lundberg et al., 1968) In in vitro tests a minimum inhibitory concentration (MIC) of 1.5 μg/ml has been found sufficient, when the most common bacteria, *H influenzae* and *Diplococcus pneumoniae* are considered (Jeppesen & Schaldemose 1970).

The purpose of this study was to determine whether or not an oral antibiotic is necessary when acute maxillary sinusitis is treated by irrigation and nose drops. Doxicycline was the chosen antibiotic because of the high incidence of *H influenzae* in the secretions.

MATERIAL AND METHOD

The study was made on 61 outpatients, 39 females and 22 males, with acute maxillary sinusitis. In 15 patients the infection was bi-

Table I. *Study material*

Treatment	No. of patients	Number of sinuses treated		
		Bacterial	Sterile	Total
Doxycycline	27	20	12	32
Placebo	34	33	11	44
Total	61	53	23	76

lateral. The mean age of the patients was 34 years. Due to complete double-blind nature of this trial the placebo group became larger than the study group (Table I). The diagnosis was based on anamnestic data, clinical examination, and irrigation findings. X-ray examination (four projections) revealed homogenous shadows in the sinuses or a fluid level could be seen. All cases of sinusitis were previously untreated. Treatment consisted of irrigation of the maxillary sinuses with 100 ml 0.9% saline once weekly nose drops (xylometazoline chloride, 0.1%) three times daily The antibiotic, doxicycline or a placebo was given orally for 6 days, 200 mg on the first day and 100 mg thereafter The examiners did not know to which one of the therapeutic schemes each patient belonged. Weekly irrigations were continued until it was considered that sinusitis was cured with respect to the clinical status, irrigation findings, and ostium function. A negative irrigation and regained ostial patency are considered as signs of recovery Secretion for bacteriological examination was withdrawn through a puncture needle under sterile conditions.

Bacteriological techniques: The specimens were cultured routinely onto blood- and Mc Leod agar plates under aerobic conditions. Boiled-meat medium was used for the detection of anaerobes. The bacteria detected were identified using methods routinely employed in the bacteriological laboratory The strains were collected and stored at −20 C in Stuart medium for several weeks until MIC-determinations were carried out in bath.

In a preliminary study the agar diffusion method using discs containing doxicycline was used, but the variation was found to be unacceptably large. Therefore throughout the study McLeod agar was chosen as culture medium, and the inoculum was adjusted to approximately 10^3 micro-organisms per ml. All determinations were performed twice. If the difference between results was more than two dilution steps, an additional control determination was performed. A later bacteriological control was not subsequently performed routinely in the subjects.

Assessment of the patency of the ostium, which included a direct pressure difference recording (Kortekangas, 1970), was performed with a puncture needle before irrigation (Drettner 1965 Rantanen & Kortekangas, 1971) The apparatus used consisted of the Elema-Schönander EMT 33 electromanometer and a Mingograph recorder According to the results the ostia were classified as open, partially open, or obstructed. An open and a partially open ostium were considered normal.

Findings at irrigation were rated macroscopically with respect to the amount and quality of the secretion purulent secretion, mucous secretion, no secretion. If no secretion appeared by irrigation or if it turned from purulent to mucoid and in addition diminished considerably in amount the result of treatment was rated as good. No attention was paid to the cytological findings or to the viscosity of the secretion.

A number of the bacterial strains found in the secretion were tested in relation to their sensitivity to doxicycline by a dilution method.

RESULT

Of the 76 sinuses (Table I) examined, bacteria were isolated from 53 (70%) (Table II) the remainder were sterile. In 40 sinuses the bacteria discovered were either *Haemophilus influenzae* or *Diplococcus pneumoniae* Mixed flora was found in only four samples. The results of the sensitivity test of the strains are given in Table III. Four of the isolated strains

Table II *Bacteria isolated from 53 sinus secretions*

Bacteria	No. of sinuses
Streptococcus pyogenes	1
Diplococcus pneumoniae	19
Haemophilus influenzae	19
Staphylococcus aureus	2
Streptococcus viridans	3
Proteus mirabilis	1
Alcaligenes faecalis	2
Escherichia coli	1
Staphylococcus albus	1
Haemophilus influenzae Streptococcus viridans	1
Diplococcus pneumoniae Haemophilus influenzae	1
Streptococcus viridans Neisseria crassa	1
Proteus mirabilis Staphylococcus albus	1
Total	53

(2 *Proteus mirabilis Escherichia coli* and *Staphylococcus albus*) showed an elevated resistance (MIC ≥ 2.5 μg/ml) against doxicycline.

From an assessment of the macroscopic changes of the secretion after 1 week's treatment (Table IV) the results were rated as good in 16/32 cases (50%) of the doxicycline group and in 21/44 cases (47.7%) of the placebo group. In the group with bacterial sinusitis a good result was obtained in 20/53 cases (35.8%) and in the group with sterile sinusitis in 17/23 cases (73.9%). There was no retention of secretion after 1 week in the doxicycline group in 10/32 cases, of which 7 had been sterile even at the first irrigation, and in the placebo group in 10/44 cases of which 2 had been sterile at the first irrigation. After 2 weeks of treatment the cumulative incidence of sinuses without secretion was 22 of the doxicycline group and 34 of the placebo group. In the 20 subjects who showed a retention of secretions after 2 weeks' treatment, a complete recovery followed during additional 2 weeks of irrigation therapy.

Neither in the doxicycline group nor the placebo group was there any correlation between recovery time and the sensitivity of the bacteria to doxicycline.

Improvement in the patency of the ostium could be observed each week in all the groups (Fig. 1). At the beginning of treatment, the ostia were obstructed in the whole series in 52 (69%) 1 week later in 27 (35.5%), and 2 weeks later in 10 (13%) sinuses. The corresponding figures for the doxicycline group were 68.8, 31.3 and 15.6% and for the placebo

Table III *MIC-values at initiation of treatment and macroscopic changes in secretion after 1 week of treatment*

44 sinuses. Doxycycline – Placebo – o

MIC μg/ml	Changes in secretion 0	1	2	3
>10				o
10		o		
5				
2.5	o			
1.25				
0.6	oo	o		o
0.3	ooooooo	ooo	o	oooo
0.15	o		oo	oo
0.07				o
0.04	×			

0 – no changes in secretion.
1 – secretion has become mucous, dispersed, ample.
2 – secretion has become mucous, consistent, scant.
3 – no secretion.

Table IV *Macroscopic changes in the quality of the secretion 1 week after diagnostic irrigation*

	Changes in secretion 0	1	2	3	Total
Bacterial inflammation					
Doxicycline	7	7	3	3	20
Placebo	12	7	6	8	33
Sterile inflammation					
Doxicycline	2	0	3	7	1
Placebo	3	1	5	2	11
Total					76

0 – continuously mucopurulent secretion.
1 – secretion has become mucous, still abundant.
2 – secretion has become mucous and scant.
3 – no more retentive secretion.

Table V *Weekly recovery progress with respect to clinical picture irrigation findings and ostium function*

76 sinuses. Percentages show weekly recovery

	Duration of treatment, weeks				
	1	2	3	4	Sinuses
Doxicycline					
Bacterial inflammation	4 (20 %)	10 (50 %)	5	1	20
Sterile inflammation	10 (83 %)	—	1	1	12
Placebo					
Bacterial inflammation	14 (42 %)	11 (33 %)	6	2	33
Sterile inflammation	5 (45 %)	6 (55 %)	—	—	11
Total	33 (43 %)	27 (36 %)	12	4	76

group 68.2, 38.6 and 11 4 %. If the grouping of the series is based on the bacteriological finding at the first irrigation without respect to oral therapy the bacterial group showed ostial obstruction in 75.5 % at the beginning of treatment, in 43 4 % 1 week later and in 16.9 % 2 weeks later. For the sterile sinuses the corresponding percentages were 52.2, 17.3 and 4.3 % respectively.

The mean recovery time did not differ in the doxicycline and placebo groups (Table V). Of the 32 sinuses in the doxicycline group, 14 had recovered after 1 week and 24 after 2 weeks. Of the 44 sinuses of the placebo group

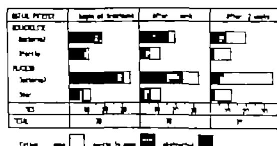


Fig. 1. Ostial patency. The frequency of different types of ostial patency at the beginning, 1 week after and 2 weeks after the commencement of therapy. In the results column, after 2 weeks, a total of open ostia is represented by an additional square.

19 had recovered after 1 week and 36 after 2 weeks.

Of the 53 sinuses with bacteria 18 had recovered after 1 week and 39 after 2 weeks. Among the 23 sterile sinuses the respective figures were 15 and 21.

DISCUSSION

The distribution of bacteria in this material agrees with previous observations. The proportion of sterile maxillary sinusitis usually varies between 20 and 40 %. In this material it was 30 %.

Daily irrigation of maxillary sinuses on consecutive days has been found to shorten the time of recovery in acute maxillary sinusitis as compared with weekly irrigation. In the material presented, the interval between irrigations was chosen to be 1 week, as it is thus easier to follow the changes in sinus secretion and opening up of the ostium. Also the positive effect of irrigation does not become too accentuated when attempting to compare the effect of drugs.

The macroscopic assessment of secretion and the changes therein is difficult and in practice large variations occur. Nevertheless, estimations are always carried out and treatment is discontinued when irrigation brings no secretion. The secretion disappears from the maxillary sinus either by reabsorption and/or by being moved through the ostium to the nasal passage by the epithelial cilia. In the latter case the ostium must be unobstructed as it is in healthy persons.

When studying the function of the ostium, one has to be satisfied with measuring its patency and resistance. Measurement of the patency using a manometer is more sensitive but it is also more prone to error. The method enables one to differentiate an open, a partially open, or an obstructed ostium, especially if simultaneous measurements are carried out via puncture needle and contralateral nostril, i.e. the pressure difference recording. How-

ever no information on the ciliary function is acquired by using this method.

In the present series the effects of doxicycline and placebo can be compared during the first week of therapy if a probable continuous *antibacterial effect is considered*, as the therapy lasted only 6 days. Despite this, we know that the half-life of doxicycline in serum is long its ability to penetrate the tissues is good and it has been found to exist in pus from maxillary sinuses in concentrations many times higher than the corresponding MIC-values (Lundberg et al., 1968). In this study sensitivity determinations could be performed in two cases on pathogenic bacteria obtained from sinus secretion immediately after doxicycline therapy

In both cases (*S aureus* and *H influenzae*) the bacteria were still sensitive to doxicycline. The reason for this discrepancy may be explained by the different microbiological methods, by a failure in the penetration of doxicycline into the sinus *secretion* by difference between conditions of in vivo and in vitro tests or by the failure to take the medicine which the patients deny In this study it was observed that the changes in purulent maxil- [illegible] sinus secretion were more distinct after [illegible] in the placebo than in the doxicycline group However the difference was not significant and evened out during the following week of treatment. Significant differences could not be observed in the first 2 weeks between the doxicycline and placebo group with respect to the patency of the ostium. On the other hand ostial obstruction was less frequent in the sterile group at the initiation of treatment and its opening up occurred faster than in bacterial sinusitis. Differences in the viscosity of pus and anatomical variations of the ostium might explain this difference.

On the basis of the experiments presented, we consider that with doxicycline the recovery time in acute maxillary sinusitis cannot be shortened, if simultaneous weekly irrigations and daily nose drops are used.

ZUSAMMENFASSUNG

61 Patienten, 39 Frauen und 22 Männer mit akuter bakterieller oder steriler maxillarer Sinusitis wurden studiert, um festzustellen, ob ein orales Antibiotikum während einer Behandlung mit Irrigation und Nasentropfen notwendig ist. Im Doppelblindversuch wurden *einem Teil der Patienten Doxicyclin und einem anderen* Teil Plasebo gegeben. Sekretionsveränderungen wurden mikroskopisch und Ostiumveränderungen rhinomanometrisch beobachtet. Signifikante Unterschiede in der Heilungsdauer der mit Doxicyclin bzw Plasebo behandelten Patienten konnten nicht festgestellt werden. Die Sensitivität der Bakterien aus dem Sekret mit Doxicyclin stimmte nicht mit der Heilungsdauer in den beiden Gruppen überein. Die Resultate ergeben, dass die Behandlungszeit von akuter maxillarer Sinusitis mit oraler Verabreichung von Doxicyclin nicht verkürzt werden kann.

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Acta Otolaryng 76 63–69 1973

NEUROGENIC LESIONS IN THE CRICOTHYROID MUSCLE IN IDIOPATHIC VOCAL CORD PARESIS

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(Received November 1 1972)

Abstract EMG recordings were made from the cricothyroid muscle in 9 patients with idiopathic vocal cord paresis. In 8 patients the paresis was unilateral; in one it was bilateral. One EMG examination was made in each case at 1–17 months after onset of symptoms, except in 2 cases in which two or three repeated examinations were made at different stages of the disease. The muscle action potentials were compared with those of healthy cricothyroid muscles and with those of the ipsilateral vocal muscle. From the cricothyroid muscle in 8 patients, potentials typical of reinnervation were encountered among some normal potentials. In one patient, all potentials were of normal type but the interference pattern was incomplete. In the vocal muscle there were signs of conduction block, denervation or reinnervation. The findings show that neurogenic lesions frequently appear both in the cricothyroid and in the vocal muscle in idiopathic vocal cord paresis.

In idiopathic vocal cord paresis, peripheral neurogenic lesions were consistently found in the vocal muscle which is supplied by the recurrent laryngeal nerve (Haglund et al., 1972). A neurogenic lesion also in the cricothyroid muscle, which is supplied by the superior laryngeal nerve, would suggest an affection of the vagal nerve, or a more general involvement of its branches, the two nerves originating from the vagal trunk and approaching the larynx by different routes. On the other hand, if the paresis were restricted to muscles innervated by the recurrent laryngeal nerve this would suggest a more localised injury along its lengthy course through the neck and thorax. Support for the existence of occasional lesions in the superior laryngeal nerve in cases of idiopathic vocal cord paresis has been given in previous investigations. Thus, electromyographic studies have shown either an incomplete interference pattern, or a diminished maximum amplitude, in a few of the cricothyroid muscles examined (Faaborg-Andersen, 1957 Hiroto et al., 1968 Dedo 1970). Recent analysis of the motor unit potentials in the normal cricothyroid muscle (Haglund, in press) gives a more reliable basis for the evaluation of pathological changes in this muscle.

In this investigation, electromyography of the cricothyroid muscles was used to determine the possible presence of lesions in the superior laryngeal nerve in a series of patients with idiopathic vocal cord paresis. For comparison recordings were also made from the vocal muscles. The results indicate peripheral neurogenic lesions in the cricothyroid muscles of 8 patients, and in the vocal muscles in each of the 9 patients. Thus, it can be concluded that in patients with idiopathic vocal cord paresis there is frequently a concomitant affection of the motor axons to the cricothyroid and to the vocal muscle.

This investigation was supported by grants from the Swedish Medical Research Council and The Paul Frenckner Fund.

Table I *Electromyographic data from cricothyroid muscles in idiopathic vocal cord paresis*

In the different columns are given side of examination, time of recovery of vocal cord mobility after onset of symptoms of paresis, time of electromyographic examination after onset, mean duration and dispersion of sample of potentials in each muscle, relative number of potentials with a duration of 2 ms or less, an amplitude of 0.15 mV or less, an amplitude equal to or larger than 0.80 mV as well as of potentials with five or more phases. The interference pattern is *given as normal, incomplete, or none. Corresponding data from normal muscles in brackets.*

Case	Side of exami-nation	Time of recovery (months)	EMG time after onset (months)	n	Duration: Mean and S.D., ms [3.99 ± 1.40]	Duration: <2 ms (%) [0–16 %]	Amplitude: ≤0.15 mV (%) [10 %]	Amplitude: ≥0.80 mV (%) [10 %]	Poly phasic (%) [0–4 %]	Interference: Pattern	Interference: Max. amp mV [1–2.0]
1	Left	7	1.5	8	6.90 ± 2.95	0	50	0	0	Incomplete	0.2
2	Left	3	–	23	5.48 ± .55	9	26	22	13	Incomplete	0.8
3	Left	4.5	.5	18	3.64 ± 1.00	10	0	5	5	Incomplete	0.8
4	Left	Nil (6)	3	7	6.29 ± .05	0	0	0	50	Incomplete	0.6
4	Right	Nil (6)	3	18	3.91 ± 1.23	6	0	17	0	Incomplete	0.7
5	Left	Nil (7.5)	3.5	34	3.61 ± 1.19	6	20	0	15	Incomplete	0.3
5	Left	Nil (7.5)	5	31	5.04 ± 1.72	3	3	36	3	Incomplete	0.8
6	Right	Nil (9)	7	.5	6.53 ± 3.01	4	16	20	16	Incomplete	1.0
7	Right	Nil (9)	7	36	5.39 ± 1.78	0	32	6	20	Incomplete	0.7
8	Right	9	7	23	6.52 ± 2.77	0	13	0	17	Incomplete	1.0
9	Left	Nil (19)	3	3	—	—	—	—	—	None	—
9	Left	Nil (19)	10	27	3.50 ± 1.31	19	7	30	11	Incomplete	1.0
9	Left	Nil (19)	17	34	5.99 ± 2.17	3	3	35	9	Incomplete	1.0

Duration and amplitudes of the potentials 5.0 ms, 100 μV 5.0 ms, 120 μV 3.75 ms, 100 μV

MATERIAL AND METHOD

The patients examined were 6 men and 3 women with an age range of 31–71 years. They were consecutive cases with idiopathic vocal cord paresis seen at the Department of Oto-laryngology at Karolinska sjukhuset from May to September 1972. The classification of the paresis as idiophatic was based on an extensive clinical investigation which failed to reveal any underlying cause. The clinical examination included laryngoscopies, X-rays of the lungs, the mediastinum, the skull and skull base, and of the oesophagus. Laboratory tests were taken of blood and urine to ascertain first the possible presence of diabetes mellitus, thyreotoxicosis or a luetic affection. In 4 of the patients, the paresis was preceded by an upper respiratory tract infection, probably viral in origin, occurring within 1 month prior to the onset of hoarseness. In 8 patients one vocal cord was immobile, while in one case there was a bilateral paresis with an immobility on one side and a decreased mobility on the other. Of the 9 patients, 4 regained normal mobility of the vocal cords 3–9 months after the onset of symptoms. The remaining 5 cases were under observation 6–19 months after the onset during which time the vocal cords remained paralysed.

The vocal and the cricothyroid muscle on the paralysed side were subjected to electromyographic examination. This examination was performed on both sides in the case with bilateral paresis. In 2 patients, repeated electromyographic examinations were made.

In previous papers descriptions have been given of the recording procedure in the vocal muscle (Knutsson et al. 1969) and in the cricothyroid muscle (Haglund, in press). Briefly, the technique implies recording of the electromyographic activity during quiet breathing, phonation and deglutition. During quiet breathing, individual potentials could usually be recorded and measurements taken of their duration, amplitude and shape. As distinct from normal muscles, a slight activation was sometimes needed to record action potentials from the cricothyroid muscles. Attempts were made to obtain a sample of motor unit po-

Table II *Electromyographic data from vocal muscles*

Patients and recordings as in Table I. Data from normal vocal muscles in brackets.

Case	n	Duration: Mean and S.D., ms [3.76±1.01]	Duration: <2 ms (%) [0–15%]	Amplitude: <0.15 mV (%) [10%]	Amplitude: >0.70 mV (%) [10%]	Polyphasic (%) [0–15%]	Interference: Pattern	Interference: Max. amp. mV [1.5–2.5]
1	2[a]	—	—	—	—	—	—	—
2	1[b]	—	—	—	—	—	—	—
3	26	4.83±1.15	0	8	70	0	Incomplete	0.4
4	40	3.96±1.40	5	5	15	20	Incomplete	0.2
4	10	2.00±0.87	50	10	20	0	Incomplete	0.6
5	12	4.23±1.85	25	17	8	58	None	—
5	19	5.58±1.43	0	5	20	15	Incomplete	0.5
6	15	2.33±1.27	53	27	20	7	None	—
7	12	4.90±1.80	0	0	70	8	Normal	1.4
8	10	2.25±0.79	30	30	10	0	Incomplete	0.4
9	9	3.25±1.08	0	10	0	0	Incomplete	0.4
9	2[c]	—	—	—	—	—	None	—
9	3[d]	—	—	—	—	—	None	—

[a–d] Duration and amplitude of potentials.
3.0 ms, 300 μV 6.5 ms, 200 μV
[b] 3.0 ms, 50 μV
5.0 ms, 300 μV 5.5 ms, 200 μV
[d] 7.5 ms, 300 μV 3.75 ms, 700 μV 5.0 ms, 200 μV

tentials large enough to permit a statistical evaluation. Owing to extensive paresis, only few action potentials could be recorded from some of the muscles. In these, further electromyographic findings made a conclusive evaluation possible.

RESULT

Data on action potentials and interference pattern from the electromyographic examinations of cricothyroid and vocal muscles are given in Tables I and II. For comparison, corresponding data from normal muscles are given in brackets at the head of each column. For cricothyroid muscles, these were obtained from the study by Haglund (in press) and refer to 338 potentials in 17 healthy muscles. In the case of the vocal muscles, the values were obtained from the paper by Knutsson et al. (1969) presenting an analysis of 469 potentials in 18 normal muscles.

The electromyographic pattern in the cricothyroid muscles

As shown in Table I, the mean duration in healthy cricothyroid muscles was 3.99±1.40 ms (S.D.). In individual muscles, the mean duration ranged from 3.11–4.67 ms. Thus, in the cricothyroid muscles in the cases with idiopathic vocal cord paresis (Table I) the mean duration of action potentials was larger than in normal muscles in the majority of the muscles examined. In only 4 of the 13 examinations was the mean duration within normal limits.

In all the muscles with increased mean duration of action potentials, dispersions were also increased. In consequence, there was an incidence of potentials, both of normal potentials and of potentials with increased duration. Those with increased duration were of several types. They were of low or normal amplitude and polyphasic shape (cases 2, 4 left, 7 and 8) or they were richly notched (cases 1 and 6). In other patients, many of the long potentials were

of increased amplitude (cases 2, 5 at 5 months, 6 and 9 at 17 months), some being so much increased that they may be regarded as giant potentials. An increased incidence of polyphasic potentials was also observed. In two cases (cases 2 and 6), polyphasic potentials of both low and high amplitude were observed. Some of the low were typical nascent potentials indicating early reinnervation while the large were mostly typical of later stages of reinnervation.

In 4 of the examinations, the mean duration was within normal limits. In the muscles of cases 4 right, 5 (at 3.5 months) and case 9 (at 10 months) there was an incidence of pathologic potentials. In the right-sided muscle of case 4 potentials of extremely large amplitude i.e. giant potentials, were observed. In case 5 nascent potentials were observed at the first examination 3.5 months after the onset of hoarseness. Six weeks later these had disappeared and giant potentials were observed instead. This change in type of potential is typical of a muscle during reinnervation as is also the change in electromyographic pattern observed in case 9 at 3 10 and 17 months after the onset of hoarseness. Thus, at the first examination of case 9 only three potentials could be observed despite every effort made to obtain an activation of the muscle. Neither was there, at this stage, any interference pattern during phonation or deglutition. At the following examination, 10 months after onset, there was an increased incidence of potentials of short duration suggesting fibrillation potentials. In addition, an increased incidence of potentials of high amplitude and polyphasic shape was then identified. At a still later stage 17 months after onset, there was no increased incidence of short potentials, but of high amplitude polyphasic potentials, which is to be expected after a reinnervation.

Consequent upon the above-mentioned findings of pathologic potentials of the types characteristically found at different stages of reinnervation there is strong evidence in favour of the view that the cricothyroid muscles in cases 1 and 2, and 4–9 were partially paretic, due to peripheral neurogenic lesions.

On the other hand, the action potentials identified in the cricothyroid muscle in case 3 did not differ significantly from the potentials in healthy muscles. The maximal amplitude (0.8 mV) during repeated deglutitions and phonations recorded at several different sites in the muscle was, however somewhat lower than in normal cricothyroid muscles (range 1.2–2.4 mV). Furthermore the interference pattern was incomplete. Thus, there might well have been a partial block in the motor nerve supplying the muscle. Since the EMG from the vocal muscle (Table II) in this subject was clearly indicative of a neurogenic lesion in this muscle, it seems a little far fetched to suggest a central lesion as explanatory of the low amplitude interference pattern in the cricothyroid. However it must be taken into consideration that the recording of a slight deviation from normal interference pattern does not in itself give unequivocal support to the presence of an injury. Even in healthy muscles, the recorded activity during phonation and deglutition may be low at certain points within the cricothyroid muscle. Consequently it is not possible to decide with certainty whether or not there was a partial nerve block.

Comparison of the EMG from the vocal and the cricothyroid muscles

In 4 of the subjects (case 4 right, case 5 at 3.5 months after onset, and cases 6 and 8) the EMG revealed an increased incidence of short potentials in the vocal muscle, indicative of the presence of fibrillation potentials, and thus of denervation. In the cricothyroid muscles in the same subjects and at the same time after the onset of symptoms, there were no signs of denervation and only normal potentials and potentials characteristic for reinnervation were observed. This fact support the view that reinnervation in the vocal muscle in these cases lagged behind the reinnervation in the cricothyroid muscle. In one of these (case 5) in which an examination was made at a later

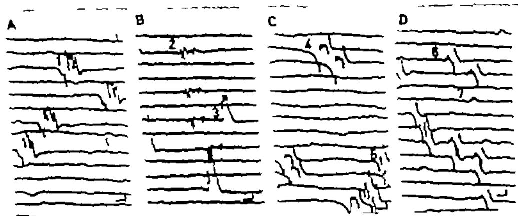


Fig. 1. Action potentials of different types (marked 1–7) recorded from vocal and cricothyroid muscles in idiopathic vocal cord paresis. Horizontal bars are 2 ms. Vertical bars in A and C are 100 µV, in B and D 50 µV.

stage (5 months as opposed to 3.5 months after onset of paresis) this difference in electromyographic pattern had vanished, both the vocal and the cricothyroid muscles showing reinnervation potentials, but lacking denervation potentials.

In 3 subjects (cases 1, 2 and 9) the activity in the vocal muscle was almost completely blocked, whereas in the cricothyroid muscles there were signs either of early or more advanced reinnervation.

In the cricothyroid as well as in the vocal muscles, polyphasic potentials of 3 different types were observed. These types are illustrated in Fig. 1. Two types of potential characteristic of reinnervation are shown in A and B. The potential marked 1 is a polyphasic potential of large amplitude and increased duration. The potential marked 2 is the low amplitude, polyphasic potential of nascent type that in cricothyroid muscle usually was of a somewhat increased duration. The third type of potential is illustrated in C and D by two potentials characterised by having one compound action potential of an amplitude and duration that are both within the normal ranges. To this is added a series of smaller waves that either follow directly after the large action potential wave (potential 4) or precede this (potential 6).

DISCUSSION

In 8 of the 9 patients an [illegible] tion of potentials was [illegible] examination of the [illegible] addition, there was an [illegible] potentials with high or low [illegible] phasic shape, as [illegible] ference pattern of low [illegible] ings show that [illegible] A myopathy is [illegible] tients, as in the [illegible] the potentials [illegible] to that of [illegible] owing to [illegible] fibres [illegible] berg, 195[illegible] plitudes [illegible] not [illegible] pattern [illegible] in the [illegible] vaguely [illegible]

[illegible] present [illegible] previous study [illegible] (Haglund et al., [illegible] neurogenic [illegible] signs of [illegible]

Thus, concomitant lesions in the superior and the recurrent laryngeal nerves seem to be a characteristic feature of idiopathic vocal cord paresis.

The findings cited above do not necessarily imply that the laryngeal muscles are subjected to a partial or complete denervation in the occasionally observed idiopathic vocal cord paresis of duration shorter than 1–2 months. In patients with idiopathic facial paresis, fibrillation potentials were observed in only 40 % of the patients, when successive electromyographic examinations were made starting within less than 2 weeks from the onset of the disorder. In the remainder conduction block was reported. The majority of these cases recovered within 2 to 3 months after the onset of the paresis (Taverner 1955 1959). Whereas patients with idiopathic facial paralysis evidently consult the physician soon after onset of the disorder the majority of the patients in this and in a preceding communication (Haglund et al. 1972) were not available for electromyographic examination until 2 months after onset of hoarseness. The possibility must be taken into consideration that there are some patients who suffer from hoarseness due to idiopathic vocal cord paresis, and who re- within a month or two without having consulted a physician since they do not find their symptoms sufficiently alarming.

A later reinnervation of the vocal than of the cricothyroid muscle was observed in 4 patients in the present study. If axon regeneration was the only mode of reinnervation this may suggest that the lesions of the motor axons are situated more remotely from the vocal muscle than from the cricothyroid. However severe injuries tend to increase the initial delay and decrease the rate of axon regeneration (Sunderland, 1947). The observed lag in reinnervation of the vocal as compared with the cricothyroid muscle may also be expected if the motor axons of the vocal muscle were more severely affected. Since the degree of injury to the motor axons of the two muscles is not known, the site of the lesions cannot be determined. Further difficulties in trying t estimate the level of the nerve injury a encountered when considering that axon r generation was probably not the only mech anism of reinnervation. In all of the two di ferent laryngeal muscles investigated, some c the recorded potentials were of normal typ which indicates that some of the motor axon were viable. Thus, the possibility exists that som of the muscle fibres were reinnervated the bifurcation of viable axons (cf Hines e al. 1945 Hoffman, 1950 van Harreveld 1952). Some indications as to the mode of re innervation might be obtained from the analys of the shape of the polyphasic potentials. Som had normal duration and consisted only of series of repetitive spikes (cf Fig. 1) suggesti that they derived from discharges occurrin exclusively in recently reinnervated musc fibres. Others were of long duration and con sisted of smooth waves, preceded or succeede by notches and spikes suggesting that the derived from a normal motor unit, which b sprouting mechanism had included previousl denervated muscle fibres (cf Fig. 1 C and D).

Previous clinical studies reveal that a voca cord paresis may be classified as idiopathic i about one-third of the cases (Williams, 1959). The present examination has demonstrated frequently occurring concomitant injury of the superior and recurrent laryngeal nerve in pa tients with idiopathic vocal cord paresis. Thes findings taken together suggest that an electro myographic examination of the vocal and th cricothyroid muscle should be performed i cases of vocal cord paresis in which the unde lying cause is unknown. Obviously there is great chance in these cases to demonstrate th presence of injuries to motor axons in bo muscles. Such findings point to the diagnos of an idiopathic vocal cord paresis, making latent malignancy affecting the recurrent ner in the lower neck or in the thoracic apert less probable.

ZUSAMMENFASSUNG

EMG-Aufzeichnungen des M. cricothyreoideus wurden an 9 Patienten mit idiopathischer Stimmbandlähmung vorgenommen. Bei 8 Patienten war die Parese einseitig, bei einem Patienten beidseitig. In jedem Falle wurde eine EMG-Untersuchung 1 17 Monate nach Beginn der Symptome durchgeführt, bei 2 Patienten hingegen 2 oder 3 Untersuchungen in verschiedenen Krankheitsstadien. Die Muskelaktionspotentiale wurden mit denen des gesunden M. cricothyreoideus und mit denen des ipsilateralen M. vocalis verglichen. Bei 8 Patienten wurden neben normalen Potentialen auch typische Reinnervationspotentiale des M. cricothyreoideus beobachtet. Ein Patient dessen Potentiale alle dem Normaltyp entsprachen, wies jedoch ein unvollständiges Interferenzmuster auf. Am M. vocalis fanden sich Zeichen von Überleitungsblock, Denervation und Reinnervation. Die Ergebnisse verdeutlichen die Tatsache, daß die neurogenen Läsionen bei der idiopathischen Stimmbandlähmung häufig sowohl am M. cricothyreoideus als auch am M. vocalis auftreten.

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Acta Otolaryng 76 70–74 1973

TRANSPLANTATION OF CANINE LARYNX AND HYPOPHARYNX WITH THYROID AND PARATHYROID GLANDS USING CONTINUOUS HYPOTHERMIC PERFUSION

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(Received September 26, 1972)

Abstract The described method of transplantation of larynx and hypopharynx with thyroid and parathyroid glands using continuous hypothermic perfusion has the following advantages.

1 The blood supply of the transplanted specimen is practically normal.

2 The continuing cold perfusion keeps the transplanted larynx in good condition and allows the surgeons plenty of time to perform the operation.

3 Transplantation of thyroid and parathyroid glands in the same specimen together with the larynx ensures that the necessary hormones will be available to the dogs during the postoperative period.

4 Transplantation of hypopharynx in the same \ together with larynx makes reconstruction of pharynx easier

5 Infusion of the donor dog's blood into the recipient helps the animal to survive the operation.

6 The separate tracheostoma keeps the pharyngeal secretions out of the trachea and lungs, thus protecting them from infection. The idea of suturing th caudal opening of the transplanted specimen aside from the midline serves the same purpose.

The methodology of canine larynx transplantation has been described in some earlier publications (Ogura et al., 1966 Silver et al. 1966)

We ourselves have performed such transplantations and recognized that the technique of canine larynx transplantation—both the principles followed and the practical performance—should be made available to others as this would facilitate the work of future investigators. It is with this in mind that we report on the method which, in our experience, has some advantages.

The dogs

We have used mongrel dogs of roughly equa size Naturally big dogs have larger bloo vessels and, since one of the main technica difficulties in larynx transplantation is pre sented by vascular suturing, it is of advantage to use fairly big dogs for this purpose.

Surgical conditions

Transplantation of the larynx is so demanding a procedure, both surgically and from the point of view of the distress caused to the animals, that all the circumstances should as far as possible be comparable to those applying to laryngeal operations on humans. The dogs must be in good general health. The conditions in the operating room as well as surgical asepsis, anesthesia, and the facilities for blood transfusions and electrolyte administration should be comparable to those under which similar operations are performed on man. In fluid administration, special regard should be had to the relatively small weight of the dog and the danger of fluid overdosage

Preoperative preparation

Anesthesia is induced with Hypnostan intravenously (preferably using a long plastic cannula which is pushed sufficiently deep into the vein and remains firmly in place during the

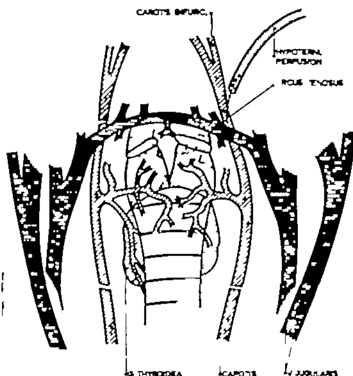


Fig. 1. The transplanted larynx specimen in the middle of the picture. The figure shows the arterial and venous anastomoses to be made to the relevant vessels of the recipient dog.

operation). *Before intubation the dogs receive* an intravenous dose of 0.5 mg of atropine. Intubation is carried out and the tracheal tube connected to the anesthesia apparatus. For breathing gas it is of advantage to use a combination of oxygen and nitrous oxide, the former in 20% concentration. Relaxation is neither necessary nor desirable in dogs. To maintain an adequate level of anesthesia, small additional doses of Hypnostan are given. For checking of acid–base balance, micro-Astrup values should be recorded at sufficiently frequent intervals and possible changes in this balance corrected. In a number of cases, sodium bicarbonate must be given towards the end of the operation to prevent acidosis.

Other general preparations

The general rules of surgical asepsis are followed. The neck is shaved sufficiently widely and the area cleaned thoroughly with Betadine. Operation towels are employed in the usual way. The dogs are placed in supine position with each leg tied separately to the corners of the operating table. If only one operation team is available, the operation is started by first dissecting free the larynx of the donor dog in preparation for its complete excision; after this is done the larynx of the recipient dog is dissected and excised. The donor larynx is then excised, kept under hypothermic perfusion, and transplantation performed. The blood of the donor dog is collected into citrate-containing transfusion bags.

Excision of donor dog's larynx

A U-incision is made so that its bottom corresponds to about 3 tracheal rings. The side limbs pass slightly lateral to the external jugular veins, extending upwards to the level of the hyoid bone. The skin flap thus formed is separated by sharp dissection and fixed with a sheet clamp to the submental region of the dog.

The external jugular vein is exposed at the level of the inferior border of the incision proceeding to the arcus venosus without, however attempting to remove wholly the membranes protecting the arcus. The veins running from the arcus to the submental area are ligated about 2 cm from the arcus, thus sparing the small branches possibly passing to the larynx to be excised. Mercilen silk 3-4-0 is used for ligature The external jugular veins are ligated immediately cranial to the branch going to the arcus to prevent the development of a venous cul-de-sac, which might cause thromboses. The muscles covering the larynx are removed, *taking care that the cranial* thyroid arteries are not injured even though the muscular branches are ligated. In this connection the thyroid gland and parathyroid are also exposed which structures are remarkably mobile and easily damaged during dissection They must be included undamaged, in the specimen because the corresponding organs of the recipient dog are also removed, and their inclusion will prevent the development of hypothyroidism and hypoparathyroidism. The common carotid artery is dissected from its bed with great care but without ...ng to remove the thin membrane covering ... artery Thick silk ligatures are now passed immediately below the bifurcation of the common carotid in readiness for its subsequent division

The approach to the pharynx in the donor dog is made between the base of the tongue and the arcus venosus working above the epiglottis. The aim is to include as much as possible of the mucosa in the cranial direction. The hyoid bone is transected and the remaining, caudal part of it (the basihyoideum) included in the specimen The mucosa is cut obliquely backward and downward, the oesophagus is cut at the border to hypopharynx, dissection being continued behind the hypopharynx and including it in the specimen. The trachea is transected leaving 6–7 cartilages attached to the specimen and attempting to leave as much as possible of the paratracheal tissue to be included. The dog is given heparin intravenously 1 mg/kg. The previously placed carotid ligatures are tied and the carotid arteries divided cranially The caudal parts of the carotid arteries are ligated or clamped The external jugular vein is clamped as low down as possible and these veins are divided without ligating them after sectioning of the arteries. Immediately after this, one common carotid artery is cannulated from the cranial end using a catheter which is fixed in position with a ligature passed round the carotid artery Hypothermic perfusion with the following composition is started.

The composition of the fluid for cold perfusion is: Haemaccel, 450 ml Gluc. 50% 10 ml Lidocaine 1% 10 ml $NaHCO_3$ 7.5% 10 ml Heparin 25 mg.

The same solution can be perfused through the contralateral carotid with a syringe. Transplantation can now be started. A sign of good effect of the perfusion is that the tissues become increasingly pale and the perfusion fluid dripping through both jugular veins rapidly becomes clear

Preparation of recipient dog

The incision is made as described above Exposure of only the main branch of the jugular vein is needed. The branch to the arcus venosus is ligated proximally on either side. The cranial thyroid artery is exposed, ligated proximally and divided bilaterally The veins passing upward from the arcus are ligated, as in the case of the donor dog The laryngeal excision differs from the one described above in that as little pharyngeal mucous membrane as possible is included in the specimen now to be removed The trachea is transected at the level of the 2nd–3rd tracheal ring.

Transplantation

During continuous hypothermic perfusion the donor larynx is transplanted to replace the recipient's larynx. The contralateral side of the perfused carotid artery of the transplant is

first anastomosed by removing from the carotid artery of the recipient, about half the length of the carotid in the vascular pedicle of the transplant. Tedvek 5-0 is used for sutures. Continuous simple sutures are made. It is advisable to flush the anastomoses with 1 50 heparin–saline solution, and also to give the recipient dog intravenous heparin, 1 mg/kg, before clamping Without removing the carotid clamps, the external jugular vein coming from the arcus is then anastomosed end-to-side to the recipient dog's jugular vein, using the same suture material. The vein should be cut obliquely to ensure good position of the transplant and good perfusion properties. *Flushing with heparin of the anastomosis* is recommended, as above A continuous suture is used again, starting from the acute angle of the anastomosis and suturing first one, then the other side. The continuous oozing of the perfusion fluid from the vein is favourable from the point of view of suturing and thus there is no reason to clamp the vein of the transplant The procedure is continued on the perfused side by first anastomosing the caudal end opposite to the perfusion cannula. Finally after anastomosing the other branch of the venous arcus, the last (cranial) end of carotis is sutured to the recipient's carotid artery At this moment the perfusion is discontinued.

A bag of blood from the donor dog is placed in drip position and the transfusion started. The clamps are removed. first the vein clamps, then the cranial clamps on the carotids, and if no massive bleeding occurs, also the caudal clamps. Minor jet-like hemorrhages usually cease rapidly and the application of a warm moist towel to the anastomoses promotes their final tightening. Blood is given according to the amount of bleeding

Suturing of the mucous membranes is started by splitting longitudinally the hypopharynx of the transplant on the dorsal side. Starting caudally at the middle, the pharyngeal mucous membranes are approximated with Mercilen silk 3-0, leaving the knots on the outside. This is continued up to the base of the tongue, and the last suture is made with chromic catgut 3-0 The divided parts of os hyoideum are stitched together using knots of chromic catgut 3-0 and the muscles between the recipient and the transplant are approximated with the same suture material and technique. The recipient's trachea is pulled out through a separate incision in the skin above the jugulum and secured to the margins of this wound. The trachea of the transplanted specimen is sutured to the skin border slightly lateral to the midline The skin flap is approximated again using knots of chromic catgut.

All four laryngeal nerves are cut during the operation. No re-anastomoses are made. During operation the dog receives penicillin in aqueous solution 5 mill. units.

Postoperative stage

Electrolytes are checked daily by recording micro-Astrup values. The physiological fluids administered per day amount roughly to 1 000 ml/15–20 kg. Heparin is given intravenously twice daily 1 mg/kg mixed with fluids. Electrolytes are given as required. Until the dog awakes and is able to turn actively it has to be turned from side to side at intervals of 2 hours or less. When required, artificial respiration (Bennett) is instituted. Maintenance of a relatively high humidity of the air is of advantage, as is the use of preparations of Bisolvon type if needed. Administration of aqueous penicillin solution intravenously is indicated for at least a week. Oedema of the jaws for 2–4 days can scarcely be avoided. From the 3rd postoperative day onward, the dog can have access to small amounts of water which can be increased if not very much of the swallowed fluid oozes out through the transplanted larynx and trachea. Antiemetics can be used to prevent vomiting. Feeding by mouth can be started when it seems suitable in each individual case The state of the larynx can be followed by inspection through the open end of the transplant The use of mild

anti-inflammatory medication may be beneficial during the first two postoperative weeks.

DISCUSSION

The above-described method of transplantation has the following advantages:

1 The blood circulation in the transplanted larynx is as physiologic as possible, the arterial blood enters the transplanted specimen from both sides through the cranial thyroid arteries and the brain's arterial supply through the carotid arteries is only temporarily blocked. The venous drainage is practically normal from the venous arch to the external jugular veins.

2 The continuing cold-perfusion keeps the transplanted larynx in good condition and gives the surgeons plenty of time to perform the operation without hurry and stress. It is also advantageous for the suturing of the anastomoses that the perfusion fluid keeps the vessel orifices open which facilitates the technical performance

3 Transplantation of thyroid and parathyroid glands in the same specimen together with larynx secures the relevant hormones for the og in postoperative period

4 Transplantation of hypopharynx in the same block together with larynx makes pharyngeal reconstruction easier

5 The infusion of donor dog's blood to the recipient dog helps the animal to survive the operation.

6 The separate tracheostoma keeps the pharyngeal secretions out of the trachea and lungs, thus protecting them from infection The idea of suturing the caudal opening of the transplanted specimen aside from the midline serves the same purpose.

Complications

We have lost dogs (1) because of excessive fluid infusion, which resulted in lung-oedema (2) because of thrombosis of one of the carotid arteries, which was caused by a too sparing use of heparin, and (3) because of infection apparently due to inadequate asepsis and sterility during operation and insufficient postoperative care.

Comment

To save dogs it would be beneficial just to exchange the dogs throats. The anatomical conditions of transplantation are then somewhat less favourable for reconstruction and of course the blood transfusion from the donor dog cannot be performed.

ZUSAMMENFASSUNG

Die beschriebene Methode der Transplantation von Larynx und Hypopharynx zusammen mit Thyreoidea und Parathyreoidea bei laufender hypothermischer Perfusion hat folgende Vorteile.

1 Die Blutversorgung des Transplantats ist praktisch normal.

2 Die laufende Kaltperfusion hält die transplantierte Larynx in gutem Zustand und gewährt den Chirurgen reichlich Zeit für die Operation.

3 Die Transplantation der Thyreoidea und Parathyreoidea im gleichen Block zusammen mit der Larynx garantiert, dass die Hunde in der postoperativen Zeit über diese notwendigen Hormone verfügen.

4 Die Verpflanzung der Hypopharynx im gleichen Transplantat zusammen mit der Larynx macht die Rekonstruktion der Pharynx einfacher

5 Die Infusion von Blut der Spenderhunde an die Empfänger trägt dazu bei, dass die Tiere die Operation besser überstehen.

6. Das besondere Tracheostom hält die Sekretionen der Pharynx von Trachea und Lungen fern und schützt diese dadurch vor Infektion. Die Suturation der kaudalen Öffnung des Transplantats seitlich von der Mittellinie dient dem gleichen Zweck.

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Acta Otolaryng 76 75-97 1973

THE PATHOLOGY OF SUDDEN DEAFNESS

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(Received January 31 1973)

Abstract. Pathological studies were performed on eight temporal bones, of which six were from individuals with unilateral sudden deafness and two from one individual with bilateral sequential sudden deafness. The hearing losses were profound in four ears, severe in three ears and moderate in one ear. At the time of onset of the sudden deafness two reported having headcolds, one had acute pharyngitis, two had pneumonia and two complained of headache. Vertigo as an associated symptom was severe in one case and mild in two cases. The principal pathological changes consisted of atrophy in varying combinations and severity of the organ of Corti, tectorial membrane and stria vascularis. These pathological alterations were judged to be more like those occurring in labyrinthitis of known viral etiology than those following experimentally induced vascular lesions in animals.

There are many known causes for deafness of sudden onset. A partial listing would include: bacterial labyrinthitis, viral labyrinthitis (mumps, measles), ototoxic drugs, temporal bone fracture inner ear concussion noise, surgical injury otitic barotrauma, inner ear hemorrhage (leukemia), macroglobulinemia, occlusion of the posterior inferior cerebellar artery (lateral medullary syndrome), multiple sclerosis, carcinomatous encephalomyelitis, glioma of the pons, metastatic neoplasms, vestibular schwannoma, Meniere's disease and severe cochlear otosclerosis. These causes for sudden deafness are abundantly documented in the literature.

There are also many cases of sudden deafness in which the etiology is not so obvious. The two most popular concepts to explain these cases are viral labyrinthitis and vascular occlusion. The advocates of these theories necessarily base their judgements on clinical observations because supporting pathological material is lacking. Other ideas, such as idiopathic rupture of the cochlear duct or involvement of the central auditory pathways seem too improbable to deserve serious thought.

In 1962, a report emanated from this laboratory (Schuknecht et al. 1962) describing the pathological findings in the ears of four individuals who experienced sudden deafness of unknown cause. The interpretation evolved at that time was that the cochlear changes were similar to those known to occur in human labyrinthitis of known viral etiology such as mumps, rubella, and morbilli, and dissimilar to those occurring in animals following experimental obstruction of the arteries or veins of the inner ear.

Since the 1962 report we have added over 700 newly acquired human temporal bones to our collection and among these are four additional specimens from individuals who experienced sudden hearing loss of unknown cause.

The total number of eight ears are from six individuals with unilateral sudden deafness and one individual with bilateral sequential sudden deafness. Four of the seven individuals were male and three were female.

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Table I.

Specimen	Sex	Age	Ear	Deafness to autopsy	Test to autopsy	Death to autopsy
1 A. P	♀	39	R	20 years	3 weeks	10 hours
2. B. H	♀	44	L	11 months	6 months	15 hours
3 M T	♂	51	R	15 months	14 months	20 hours
4 B. W.-L.	♀	52	L	33 years	20 years	Unknown
5 B. W.-R.	[illegible]	65	R	20 years	20 years	Unknown
6. H H.	♂	59	R	9 days	6 days	6 hours
7 P C.	♂	63	L	4 years	4 years	13 hours
8 G S.	♂	68	L	19 years	19 years	8 hours

Their ages at the time of onset of sudden deafness ranged from 39 to 68 (average 55) years.

The distribution between right and left sides was equal (four each) The time span from deafness to death varied from 9 days to 33 years, the interval between audiometric test and autopsy ranged from 6 days to 20 years, and the delay from death to autopsy varied from 6 to 20 hours (see Table I).

Our studies of these ears include graphic reconstruction of the cochleae of both ears of each individual. Thus changes in the various cytological elements of the sense organs as well as the cochlear neurons, are plotted as a function of distance along the cochlear duct. By recording the spacial distribution of pathological changes in the cochlea comparison with the opposite ear is facilitated and more accurate correlations can be made with the audiometric findings. The significant data for each ear is presented in charts which show the audiogram above and charts of cochlear pathology below The frequencies of the audiogram are plotted on the anatomical frequency scale, that is, their locations along the abscissa are plotted in accordance with their known areas of greatest physiological response along the cochlear duct. The lower scale indicates distance along the cochlear duct beginning at the basal end and the black filling in the charts shows per cent of elements missing as based on estimates agreed upon by the authors

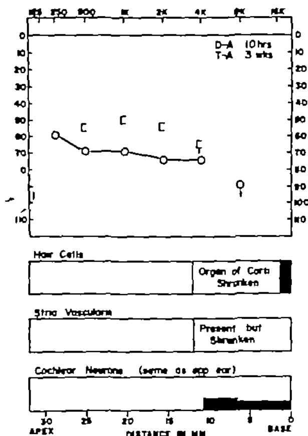


Fig 1 Ear 1 Case A.P Audiogram and cochlear chart of the right ear of a woman who at the age of 39 years experienced sudden right hearing loss while suffering from a headcold. *D–A* = time interval between death and autopsy and *T–A* the time lapse between the test of hearing and autopsy

CASE HISTORIES

Ear 1 Case A.P

At the age of 39 while suffering from a headcold, this woman awakened in the morn-

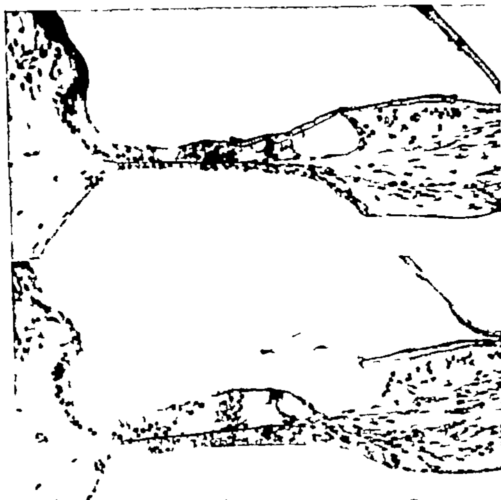
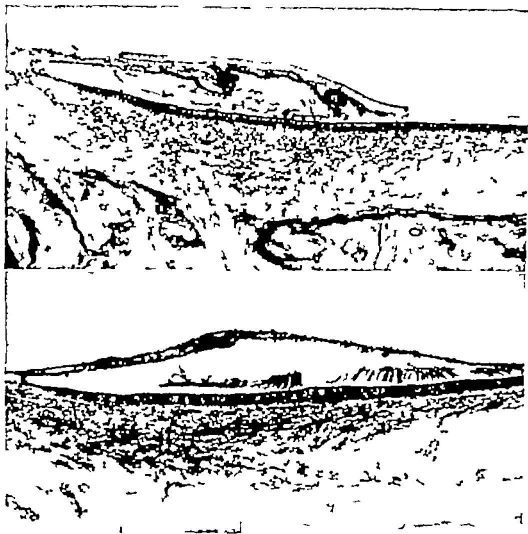


Fig. 2. Ear 1 Case A.P Photomicrographs of the organs of Corti in the 9 mm regions of the right ear above and the normal left ear below The organ of Corti and tectorial membrane of the right ear are shrunken. To accomplish rapid fixation, both middle ears were injected with 10% formalin 30 minutes after death.

ing to discover that she had a hearing loss and tinnitus in her right ear These symptoms persisted and 20 years later at the age of 59 audiometric studies were formed while she was hospitalized for terminal diffuse carcinomatosis. There was a 60 to 70 dB combined sensorineural and conductive hearing loss in the right ear (Fig. 1). Loudness recruitment (BLBT) was complete for 250 Hz and 1 000 Hz and incomplete for 4 000 Hz. The left ear showed a 20 to 25 dB hearing loss characterized by a flat audiometric pattern. Death occurred 3 weeks later

Histological study of the right ear shows shrinking of the organ of Corti and stria vascularis in the basal 12 mm of the cochlea although all cytological elements, including the hair cells, can be identified throughout the cochlear duct (Fig. 2). The tectorial membrane is also shrunken throughout the cochlea. Reissner's membrane is in the normal position. There is a slight loss of cochlear neurons in the basal turn, similar to the opposite ear

The wall of the saccule is ruptured and collapsed onto a fine network of fibrous tissue which lies between it and the macula (Fig. 3) There is a loss of more than 90%



3 Ear 1 Case A.P Photomicrographs of the saccules of the right ear above and normal left ear below In the right ear the wall of the saccule is ruptured and collapsed. Most of the hair the macula are missing. There is a network of tissue between the macula and the saccular wal

of the hair cells of the saccular macula. The utricular macula and the cristae appear normal.

The left ear shows normal sensory structures throughout. Both ears show a small focus of otosclerosis at the anterior margin of the oval window with a small area of fibrous fixation of the footplate of the stapes (Fig. 4). The cochlear endosteum is not involved by the otosclerosis in either ear

Comment

Sudden unilateral sensorineural hearing loss occurring in association with acute rhinitis is suggestive of a viral etiology The hearing loss is probably accounted for atrophic changes in the organ of Cor stria vascularis in the basal turn an ticularly by the atrophy of the tectorial brane which has occurred throughout cochlear duct. Degeneration of the sa in association with the cochlea suppo viral etiology for cochleosaccular degener is a characteristic finding in deafness of k viral etiology such as rubella and mumps (l say et al., 1953 Lindsay et al., 1960) It s doubtful that the otosclerotic focus is of si cance in the pathogenesis of the sudden he



Fig 4 Ear 1 Case A.P Otosclerotic focus at the anterior margin of the oval window of the right ear The endosteum of the cochlea is not involved. A similar lesion is present in the contralateral ear

loss because the focus is small and does not involve the cochlear endosteum.

Ear 2 Case B H

At the age of 43 this woman complained of a sore throat. Examination revealed "white patches" in the pharynx and she was treated with Gantricin. Two weeks after the onset of the pharyngitis she awakened in the morning and experienced unsteadiness and a spinning sensation which was aggravated by head movement Two days later she noticed a hearing loss and tinnitus in her left ear When audiometric tests were performed 4 months later the left ear showed a 40 dB sensorineural hearing loss for the low frequencies and a profound loss for frequencies above 1 000 H_ by both conventional and Békésy audiometry (Fig. 5). Loudness recruitment (BLBT) was incomplete at 2 000 Hz. Békésy audiometry showed a type I pattern and the speech discrimination score (PB max) was zero for that ear

The opposite ear showed a sensorineural hearing loss of about 30 dB characterized by a flat audiometric pattern and speech discrimination of 88% She exhibited slight ataxia but no nystagmus, and cold caloric responses were symmetrical and considered to be normal. The patient had been ill for many years with ulcerative colitis and, in spite of medical and surgical treatment she died of peritonitis at the age of 44 11 months after the onset of sudden deafness in the left ear

Histological study of the left ear shows the organ of Corti to be severely atrophied in the basal 11 mm of the cochlea (Fig. 6) This is the only finding which is different from the opposite ear Both ears show a normal position fo Reissner's membrane, patchy atrophy of the stria vascularis in the apical halves of the cochleae, shrinkage of the tectorial membrane and slight loss of cochlear neurons in the middle parts of the basal turns. The vestibular sense organs appear normal in both ears.

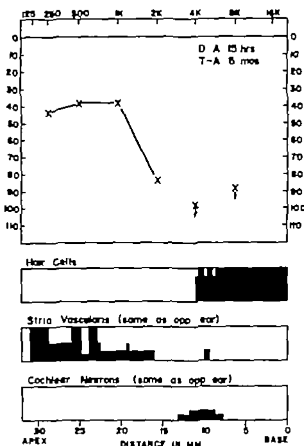


Fig. 5 Ear 2, Case B H Audiogram and cochlear chart of the left ear of a woman who at the age of 43 years experienced sudden left deafness during an episode of acute pharyngitis.

Comment

It is not possible to determine what role if any the pharyngitis played in the etiology of the sudden deafness. The occurrence of vestibular and auditory symptoms in the absence of other neurological manifestations, as well as atrophy of the organ of Corti in the basal turn implicate a labyrinthine rather than a central etiology for the sudden deafness. The elevation of thresholds for pure tones is adequately explained by the atrophic changes in the organ of Corti and stria vascularis. The pathological basis for the zero speech discrimination score is not obvious.

Ear 3 Case M T

Following a left pneumonectomy for carcinoma at the age of 55 this man suffered from chronic respiratory insufficiency At the age of 63 he developed a nasopharyngitis and was hospitalized in acute respiratory distress. Treatment consisted of antibiotic medication, a tracheotomy and use of a mechanical respirator At this time he noticed hearing loss and tinnitus in his right ear He denied experiencing vertigo at any time. Audiometric study 6 weeks later revealed a profound hearing loss in the right ear (Fig. 7) and a moderate combined sensorineural and conductive hearing loss in the left ear He could not hear a loud shout in the right ear when the left was masked with a noise box. Caloric tests using 5 cc of water at 80°F gave no response on the right and a normal response (85 sec) on the left. He died of respiratory insufficiency and leukemia 14 months after the sudden right hearing loss.

Histological study of the right ear shows severe atrophy of the organ of Corti, consisting of flattening of the cell mass, severe loss of hair cells, collapse of pillars and distortion of Hensen's and Deiter's cells. The limbus is atrophied in the basal turn. Reissner's membrane is collapsed onto remnants of the organ of Corti in the basal turn and is in the normal position elsewhere. Mild atrophy of the stria vascularis in the apical region and of the cochlear neurons in the basal turn is the same as in the opposite ear The vestibular nerves and sensory structures appear normal.

The left ear shows atrophy of the organ of Corti at the extreme basal end of the cochlea consistent with sensory presbycusis. The vestibular sense organs and nerves appear normal.

Each ear shows two identical small foci of otosclerosis, one involving the posterior part of the footplate with a small area of ankylosis to the oval window margin (Fig 8), and the other involving the margin of the round window

Comment

It seems probable that the acute upper respiratory infection is etiologically related to the

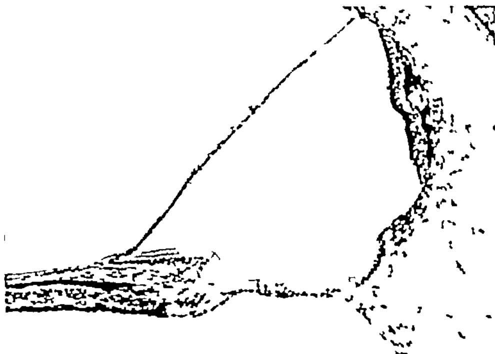


Fig. 6. Ear 2, Case B.H. The cochlear duct in the 9 mm region of the left ear. The organ of Corti has been reduced to a small mound of undifferentiated cells. The shrinkage of the tectorial membrane is the same as the opposite ear and therefore represents preparation artifact. The stria vascularis is normal in this region.

sudden hearing loss. The profound hearing loss is adequately accounted for by the severe atrophic changes in the organ of Corti. The diminished caloric response cannot be explained on a histological basis. The otosclerotic foci are probably of no etiological significance to the sudden hearing loss as they do not involve the cochlear endosteum.

Ears 4 and 5. Case B.W. (Courtesy of Professor Luzius Rüedi)

According to available history at the University of Zürich this woman was born in 1856 and could hear well in both ears until 1908 when, at the age of 52, she suddenly lost the hearing in her left ear. Subsequently she noted some degree of progressive hearing loss in her right ear but could hear fairly well until 1921 when, at the age of 65 she awakened at 2 a.m. and noticed a total loss of hearing in her right ear associated with tinnitus, vertigo and headache. Examination on the following day showed that she could not hear a shouted voice or shrill whistle in either ear. In 1941 at the age of 85 she was hospitalized at the Kantonspital, University of Zürich for a fractured femur incurred in an accidental fall. Otological examination at the time by Franz Escher (now Professor and Chairman of the Department of Otolaryngology, University of Berne, and current President of the Collegium ORLAS) revealed bilateral severe sensorineural deafness. She died 2 months later and the temporal bones were removed for histological study.

In the left ear the organ of Corti is totally missing in the basal half of the cochlea (Fig. 9). In the apical half the supporting cells are well preserved and at the extreme apex a few inner hair cells can be identified. There is severe atrophy of the stria vascularis and only isolated areas of strial tissue remain in the apical half of the cochlea. The tectorial membrane appears normal and Reissner's

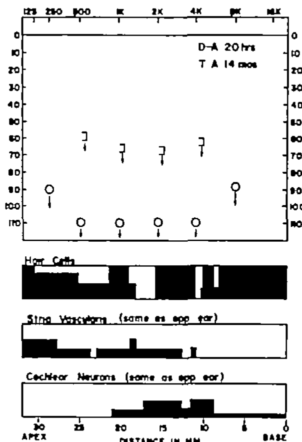


Fig 7 Ear 3 Case M.T Audiogram and cochlear chart of the right ear of man who at the age of 55 years developed sudden deafness in his right ear in association with an upper respiratory infection.

membrane is in the normal position About 10% of the cochlear neurons remain in the basal 20 mm of the cochlea and 30 to 60% in the apical region.

In the right ear the organ of Corti is missing in part of the basal turn but in the remainder of the cochlea the supporting cells are well preserved and about 50% of the hair cells can be identified (Figs. 10 and 11). There is patchy atrophy of the stria vascularis in the apical half of the cochlea. The tectorial membrane appears normal. About 10% of the cochlear neurons remain in the basal 8 mm of the cochlea, 30% between 8 and 17 mm and 60% in the remaining area.

Both ears. In both ears the saccules and reuniting ducts are about twice normal size whereas the size of the cochlear ducts, utricles and canals is normal. The saccular maculae as well as the other vestibular sense organs and nerves appear normal. Both ears also show large patent cochlear aqueducts (Fig. 12) The loss of cochlear neurons is nearly symmetrical and may be the result of primary neural atrophy of aging.

Comment

This patient experienced bilateral sequential sudden deafness with an interval of 13 years. Although historical detail is incomplete, it is known that she had headache on the occasion of the hearing loss in the second (right) ear She had no known systemic diseases and survived 20 years following the hearing loss in the second ear In the left ear the severe atrophy of the organ of Corti is adequate to explain the hearing loss. In the right ear however where nearly 50% of the hair cells remain, it must be assumed that the functional capability of these remaining cells was impaired. The finding in these ears of similar anatomical variants leads to speculation regarding their possible etiological significance for the bilateral sequential sudden deafness. In this regard, the large cochlear aqueducts may be of significance, for it is conceivable that viral particles circulating in the cerebrospinal fluid would find a more ready access to the inner ears.

Ear 6 Case H H (Courtesy of Dr Jan Beekhuis)

At the age of 57 this man began having severe episodes of vertigo with nausea and vomiting associated with a progressive loss of hearing in his left ear Vestibular and auditory tests as well as the history were typical for left Menière s disease. He received a variety of commonly used medications while under the care of Dr Jan Beekhuis but continued to have frequent severe vertiginous episodes.

One year after the onset of these symptoms, at the age of 58, he suddenly developed severe hearing loss in the contralateral (right)



Fig. 8 Ear 3 Case M.T There is a small otosclerotic focus in the superior margin of the footplate and adjacent margin of the oval window in the right ear The lesion does not involve the endosteum of the cochlea. A similar lesion was present in the opposite ear

ear He awakened at 4 a.m., noticed that he could not hear went to sleep and awakened again at 6 a.m. to note that he still could not hear and had a roaring noise in the right ear He stated that he had the feeling of having a headcold for the previous 3 days. Examination that day revealed normal tympanic membranes, nose and throat. Pure tone audiometric studies showed a severe sensorineural hearing loss in the right ear (Fig. 13), as well as the previously recorded moderately severe sensorineural hearing loss in the left ear due to Menière's disease. He was hospitalized and given anticoagulant heparin therapy and intravenous histamine. Neurological examination was negative. Prothrombin time was 21 4 sec (control 13.6), blood cholesterol 268 alkaline phosphatase 6.0 bromsulfthalein retention 15% serum bilirubin 1 4 blood glucose 152, cephalin flocculation negative after 24 hours, thymol turbidity 10 units, total proteins 7 6 albumin 6.2, globulin 1 4 AG ratio 4 4/1 0, and bilirubin negative. The glucose tolerance test gave the following results: Fasting, 127 3/4 hour 230· 1 hour 210 1½ hours, 120· 3½ hours, 75

Audiometric studies 3 days later showed no improvement in hearing. Caloric tests with 5 cc of ice water elicited a very minimal response on the right side and a slightly reduced response on the left side

Anticoagulant therapy was continued. Histamine therapy was discontinued on the fifth day

On the 7th day after the onset of the sudden deafness in the right ear he complained of severe pain in the left leg. Examination showed absence of arterial pulsations in that leg and an immediate left femoral embolectomy and left lumbar sympathectomy were performed. He responded well for 24 hours after which his blood pressure showed a progressive fall and he died 2 days after sur

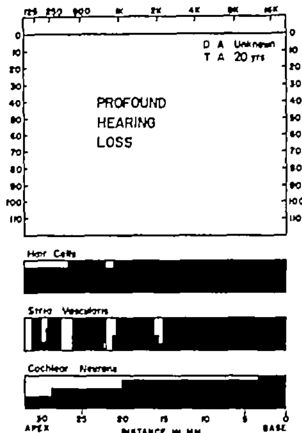


Fig 9 Ear 4 Case B W This woman sustained a profound hearing loss of sudden onset in the left ear at the age of 52 years. The opposite ear was similarly involved 13 years later (See Fig. 10)

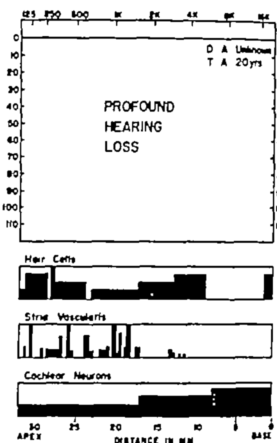


Fig 10 Ear 5 Case B.W At the age of 65 years this woman awakened at 2 a m. to discover profound hearing loss in the right ear associated with tinnitus, vertigo and headache She had experienced a sudden hearing loss in the left ear 13 years previously (See Fig 9)

ers (9 days after the onset of sudden deafness in his right ear)

Autopsy revealed bilateral basilar bronchopneumonia and thrombosis of the splenic artery There was no significant cardiac pathology

Histological study of the right ear shows a localized area of severe shrinkage of the organ of Corti and stria vascularis in the 10 to 16 mm region of the cochlea. The border between the shrunken area and normal structures is abrupt Although the cells of the organ of Corti are clumped tightly together in this area all cytological elements, including hair cells, can be identified (Fig 14) In this area the stria vascularis is shrunken to about 25% of its normal thickness. It appears as a basophilically stained ribbon within which no cytological structures can be clearly identified (Fig. 15) In the remainder of the cochlea the organ of Corti and stria vascularis show moderate swelling artifact characteristic of post mortem autolysis The spiral ligament appears normal for age throughout and is the same as in the opposite ear In the basal 15 mm of the cochlea the tectorial membrane appears as a small homogeneous sphere partly encased in a dark staining material and displaced onto the limbus, and in the remainder of the cochlea it is missing entirely There is a slight loss of cochlear neurons in the middle part of the basal turn which is the same as in the opposite ear The vestibular sense organs and nerves appear normal



Fig. 11. Ear 5. Case B.W. The top view shows loss of hair cells and severe atrophy of the stria vascularis in the 18 mm region of the right cochlea. The lower view shows total loss of the organ of Corti, a severe loss of cochlear neurons, and a normal stria vascularis in the 9 mm region. The tectorial membrane is normal throughout.

The left ear shows severe endolymphatic hydrops characteristic of Menière's disease.

Comment

This man with unknown and untreated diabetes mellitus developed femoral and splenic artery thromboses leading to his demise, raising the question of a vascular occlusive mechanism for the sudden hearing loss occurring 9 days before death. This hypothesis seems improbable however for the following reasons: (1) The atrophic change in the organ of Corti and stria vascularis extends over a 6 mm region of the cochlear duct (10 to 16 mm) which according to the studies of Nabeya (1923) would encompass an area sup-

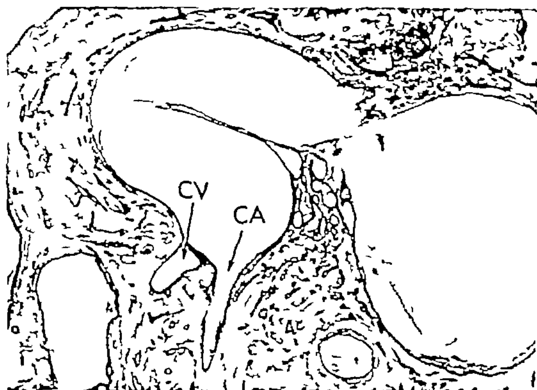


Fig 12 Ear 4 Case B W View through the basal turn of the left cochlea showing the cochlear aqueduct (*CA*). Both cochleae of this patient with bilateral sequential sudden deafness had wide patent cochlear aqueducts. The vein at the cochlear aqueduct (inferior cochlear vein) is normal (*CV*).

plied by about six external radiating arterioles. In man there are no known arteries this region of the cochlea which distribute a group of radiating arterioles but rather radiating arterioles originate directly from the main cochlear artery (2) The spiral ligament which is supplied by the same radiating arterioles as the stria vascularis is normal for age and appears histologically similar to the opposite ear (3) Recent studies (Lawrence 1966) have suggested that the organ of Corti is supplied by the system of internal radiating arterioles which distribute through the osseous spiral lamina and their terminal capillaries at the tympanic lip and basilar membrane Thus, it does not appear that occlusion of any single artery or group of internal or external radiating arterioles could explain the atrophic changes of both organ of Corti and stria vascularis, as occurred in the 10 to 16 mm region of the cochlea of this individual. The diminished caloric response in the right ear cannot be explained on a morphological basis. In the final analysis the findings seem to favor a viral etiology for the sudden deafness in the right ear particularly in view of the patient s statement that he felt he had a headcold for 3 days prior to its onset.

Ear 7 Case P C

At the age of 63 this man experienced a sudden loss of hearing in his left ear associated with severe vertigo nausea and vomiting There is no history of any associated illness The vertigo subsided in a few days but the severe left-sided deafness persisted. He received an otological examination 6 weeks later when it was determined that the tympanic membranes were normal, the cold caloric response (5 cc at 80 F) was diminished in the left ear (45 sec) and normal in the right (90 sec) and that he was profoundly deaf in the left ear There were no responses at any fre-

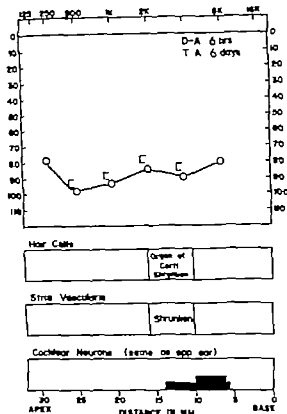


Fig. 13 Ear 6 Case H.H. Audiogram and cochlear chart of the right ear of a man who at the age of 58 years awakened at 4 a.m. to notice hearing loss and tinnitus in his right ear He stated that he had a headcold for 3 days prior to onset of deafness. He died of femoral and splenic artery thrombosis 9 days later

quency at maximum output of the audiometer for bone and air conduction (Fig. 16) In the opposite (right) ear he exhibited a hearing loss for frequencies above 4 000 Hz. He was not examined again and died 4 years later at the age of 67 of gastrointestinal bleeding, complicating Laennec's cirrhosis and cardiac failure.

Histological examination of the left ear shows the organ of Corti to be missing or flattened to a small epithelial mound in the basal 13 mm of the cochlea (Fig. 17) From the 13 mm area to the apex the supporting cells are present but about 50% of the external hair cells are missing (Fig. 18). There is a diffuse atrophic change in the stria vascularis with about 90% missing in the basal 4 mm, 50% from 4 to 21 mm and 80% from 21 mm to the apex. The tectorial membrane appears normal throughout and Reissner's membrane is in the normal position. The vestibular sense organs and nerves appear normal. The opposite (right) ear exhibits a mild loss of hair cells in the basal turn of the cochlea and a normal stria vascularis.

Both ears show a severe loss of cochlear neurons, with about 70% missing in the basal turn and 50% in the middle turn. The spiral ligaments are normal for age. Both ears have large patent cochlear aqueducts (Fig. 19).

Comment

The sudden deafness in the left ear occurred when the patient was apparently in good general health and without evidence of associated acute respiratory infection or other disorder The atrophic changes in the cochlea involve principally the organ of Corti and stria vascularis, and are adequate to explain the severe hearing loss. No morphological explanation can be found for the diminished caloric response in the left ear The severe loss of cochlear neurons occuring in both ears is presumed to represent primary neural degeneration of aging.

Ear 8 Case G.S.

At the age of 68 this man was hospitalized for a disorder which was diagnosed clinically as "viral pneumonia" During this admission he noted a hearing loss in both ears associated with intense "buzzing" The left hearing loss progressed and he developed a constant headache, radiating from the temporal to the parietal and occipital areas. He had no vertigo nausea or vomiting. The right hearing loss improved but the loss on the left persisted and 2 months later he had his first otological examination. The tympanic membranes appeared normal. Audiometric tests showed a severe combined conductive and sensorineural hearing loss on the left (Fig. 20) and high frequency sensorineural hearing



Fig. 14. Ear 6. Case H.H. Photomicrographs of the organ of Corti of the right ear. The upper view from the 20 mm region shows all cytological elements to be present but severely swollen, which would be consistent with post-mortem autolysis. The tectorial membrane is missing. The lower view from the 12 mm region shows shrinking of the organ of Corti with all cytological elements present. The tectorial membrane is distorted into a sphere displaced onto the limbus and partly encapsulated in a dark-staining substance.

loss on the right. No further otologic examinations were performed and he died 19 years later at the age of 87 of anemia, duodenal ulcer complications of hemigastrectomy and cachexia.

In the basal 13 mm of the left cochlea the organ of Corti is either missing or consists of a small mound of cells (Fig. 21). In the remainder of the cochlea many of the pillar cells and some of the Deiter's cells are missing; however the hair cell population appears to be normal. The stria vascularis is severely atrophied throughout with only small patches of strial tissue remaining. The tectorial membrane is also atrophied throughout. In the middle and apical turns it is displaced into the inner sulcus and encapsulated, in *the basal turn it is displaced onto the* limbus. Reissner's membrane is in the normal position. There is a small area of disruption of sensory epithelium of the saccular macula. The other vestibular sense organs and the vestibular nerves appear normal.

The opposite (right) ear shows atrophy of

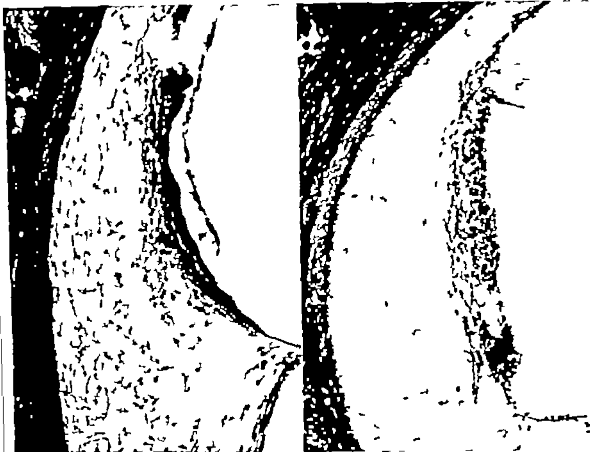


Fig. 15. Ear 6. Case H.H. Views of the stria vascularis of the right ear. In the 20 mm region (right view) the swollen tissue is consistent with post-mortem autolysis. In the 12 mm region (left view) the stria appears as a deeply basophilically staining ribbon with no clearly identifiable cytological structure. The appearance of the spiral ligament in both views is judged to be normal for age.

the organ of Corti and loss of hair cells in the basal turn and partial atrophy of the stria vascularis at the basal and apical ends of the cochlea only.

Both ears show a loss of more than 90% of the cochlear neurons throughout the cochleae (Fig. 22).

The cochlear aqueducts of both ears are small but patent throughout.

Comment

This history of onset of sudden deafness in the left ear while suffering from "viral pneumonia" associated with headache is suggestive of a viral labyrinthitis. The atrophic changes in the organ of Corti, stria vascularis, and tectorial membrane adequately explain the hearing loss. The severe loss of cochlear neurons found in both ears is presumed to represent primary neural degeneration of aging.

FINDINGS

The hearing losses were profound in four ears, severe in three ears and moderate in one year. Vertigo as an associated symptom was severe in one case, mild in two cases, absent in four cases, and the history regarding vertigo was non-contributory in one case. Six individuals noted the onset of tinnitus in association with the sudden hearing loss and

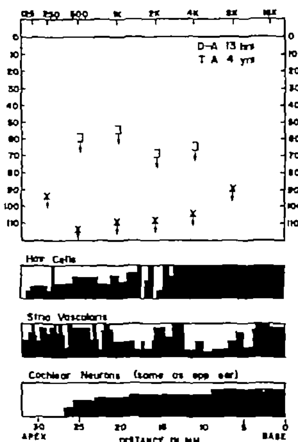


Fig 16. Ear 7 Case P C. Audiogram and cochlear chart of the left ear of a man who at the age of 63 years experienced a sudden hearing loss in his left ear associated with severe vertigo nausea and ng. There was no history of an associated ill-

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in two cases the histories regarding tinnitus were non-contributory A summary of these findings is shown in Table II.

The most spectacular and consistent pathological changes in these eight ears is atrophy of the organ of Corti. In four cochleae these changes are limited to the basal turns, wi two showing shrinkage of the organ of Cor without loss of hair cells. The other four coc leae exhibit severe atrophy of the organ Corti in all three turns, with three show changes of decreasing severity from basal apical end.

The tectorial membrane is atrophi throughout the cochlear duct in three ea In one it is shrunken and in two it is atr phied, displaced into the inner sulcus or on the limbus, and encapsulated. The stria va cularis shows atrophic changes greater th those of the opposite ear in five cases, bei shrunken throughout in one, severely atr phied in three and partially atrophied in on The six ears from individuals with unilater sudden deafness show the cochlear neuron population of the involved ear in each ca to be the same as the opposite ear The ne ronal *deficits*, which are *mild* in four ea and severe in two ears, logically evolve degenerative changes of aging which are u related to the sudden deafness. A summa of these findings is shown in Table III.

In all of the *specimens* it is possible visualize the external and internal radiati arterioles, capillaries of the spiral ligament, ca pillaries of the stria vascularis (except in area where the latter is atrophied) and limbus, a well as the vessels of the tympanic lip and basilar membrane. Among the venous cha nels which can be clearly identified in all ea are the collecting venules of the scala tym pani, the anterior and posterior spiral vei and the vein at the cochlear aqueduct.

Table II

Specimen	Hearing loss	Vertigo	Tinnitus	Associated disorders
1 A. P	++	−	+	Headcold, otosclerosis
2. B. H	+++	+	−	Acute pharyngitis, ulcerative colitis
3. M T	++++	−	−	Pneumonia, otosclerosis
4. B. W L.	++++	?		?
5. B. W-R.	++++	+	+	Headache
6. H. H.	+++	−	+	Headcold, femoral and splenic thrombosis
7 P C.	++++	+++	?	?
8. G S.	+++	−	−	Pneumonia, headache



Fig. 17 Ear 7 Case P C. This view from the 10 mm region of the left ear shows the organ of Corti to be totally missing. The tectorial membrane is normal.

In some of the specimens the arteries and veins contain blood cells and in others they do not. In no case is there obliteration or atrophy of vascular channels except for loss of the capillary network in association with atrophy of the stria vascularis.

Large patent cochlear aqueducts exist in both ears of two cases (B W and P C.). One of these cases (B W.) also has large saccules and reuniting ducts in both ears, although the size of the cochlear ducts, utricles and membranous semicircular canals are normal. None of the eight ears show fibrous tissue or bone within the labyrinth and none show

Table III

Specimen	Organ of corti: Hair Cells	Organ of corti: Supp. Cells.	Tect. Mem.	Stria	Cochlear neurons	Other changes
1 A P		+ + +	+ + +	+		Atrophy of saccule, otosclerosis
2 B H		+				
3 M T	+ +	+ + +				Otosclerosis
4 B. W -L.	+ + +	+		+ + +	+	Large cochlear aqueduct, saccular enlargement
5 B W R.	+ + +	+				Large cochlear aqueduct, saccular enlargement
6 H. H.		+	+ + +	+		
7 P C.	+ + +	+ +		+ + +		Large cochlear aqueduct
8. G S	+	+ + +	+ + +	+ + +		

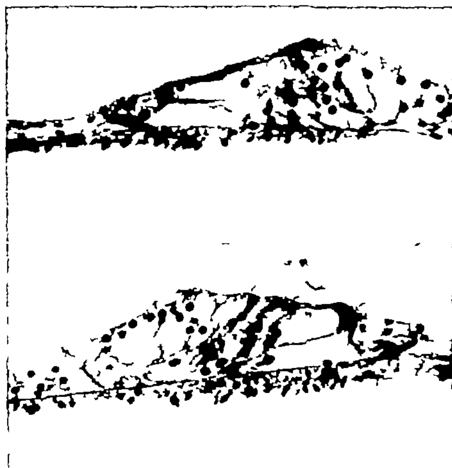


Fig 18. Ear 7 Case P.C. The upper view is from the 22 mm area of the left cochlea and shows a partial loss of hair cells. The lower view for comparison is from the 22 mm region of the right and shows normal hair cells.

y of the spiral ligament Reissner's or basilar membrane greater than to be expected for age

PROBABLE ETIOLOGY

Lindsay & Zuidema (1950) studied the clinical features of sixteen cases of sudden deafness and found that four were associated with systemic disease and twelve were unexplainable They concluded that the high incidence of the disorder in healthy adults under the age of 30 argues against a vascular etiology

Although it has been established that vascular lesions can produce sudden deafness, it is our experience that these cases occur in association with known systemic vascular disease. For example, massive inner ear hemorrhage has been identified as a cause for sudden deafness in leukemia (Schuknecht et al. 1965). Sudden deafness may also occur in Buerger's disease (thrombo-angiitis obliterans) (Kirikae et al. 1962) macroglobulinemia (Ruben et al., 1969) and disorders characterized by hyperviscosity of the blood serum (Solomon & Fahey 1963 Wilkinson et al. 1966)

Vascular disease was present in only one of the seven patients in this report. This individual (H.H) died of femoral and splenic thrombosis 9 days following the sudden deafness. It should be noted however that he also complained of a headcold at the time of onset of deafness.

We have re-examined the temporal bones from several animal experiments with induced vascular lesions in a search for similarities or differences in the pathological appearance to those observed in the eight human ears with sudden deafness. Following temporary

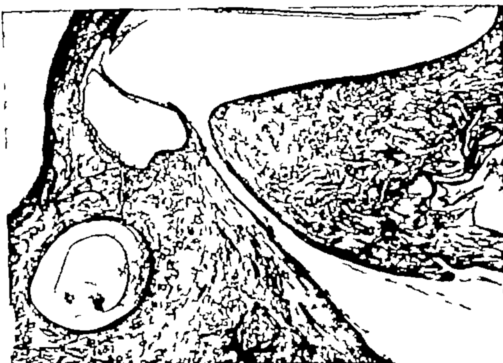


Fig. 19. Ear 7. Case P.C. This photomicrograph shows the wide patent cochlear aqueduct in the left ear. The same condition was present in the opposite normal ear.

obstruction of the labyrinthine artery (Perlman et al. 1959) there are several pathological changes which are distinctly different from those of the sudden deafness ears. The animal ears show: (1) a greater loss of cochlear neurons, (2) greater atrophy of the spiral ligament, (3) variable special pattern of hair cell loss in the organ of Corti, and (4) little effect on the tectorial membrane.

Permanent obstruction of the labyrinthine arteries of animal ears (Kimura & Perlman, 1958) produced diffuse degeneration of the membranous labyrinth. An orderly sequence of pathological changes occurred beginning in ½ hour with hair cell changes, followed in a few hours by degeneration of the supporting structures and finally in 6 months by fibrous tissue invasion and ossification of the inner ear spaces. None of the human cases showed fibrous or bony proliferation in the inner ear.

Obstruction of the vein at the cochlear aqueduct and its collaterals (Kimura & Perlman, 1956) resulted in engorgement of the vascular system and scattered hemorrhages followed by progressive loss of outer hair cells, severe atrophy of the stria vascularis, and mild atrophy of the spiral ligament, but no changes in the tectorial membrane. The late pathological changes following venous obstruction, while bearing some resemblance to the human ears, differ in that the stria vascularis is consistently severely atrophied and the tectorial membrane is consistently spared. Furthermore, we have found no evidence of venous pathology in the human ears. The various venous tributaries, as well as the two principal veins which drain the labyrinth, the vein at the cochlear aqueduct (also termed the inferior cochlear vein) and the vein at the vestibular aqueduct appear patent and normal.

Virus infection has commonly been implicated as an etiological factor for sudden deafness. The labyrinth may become involved in the course of specific viral infections such

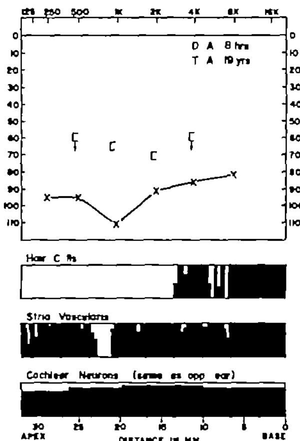


Fig 10 Ear 8 Case G.S. Audiogram and cochlear chart of the left ear of a man who at the age of 68 years experienced a severe loss of hearing in his left while hospitalized for "viral pneumonia"

measles, mumps and infectious mononucleosis (Gregg & Shaeffer 1964). It is also probable that sudden deafness may be caused by viral infection without clinical evidence of systemic involvement, for example, sudden deafness occurring in apparently healthy children or adults may be due to a subclinical mumps infection. During a mumps epidemic a considerable number of healthy contact individuals will show positive serological tests (Wolff 1953) Van Dishoek & Bierman (1957) performed serological tests and viral cultures of the blood and stools on sixty-six patients with sudden severe unilateral hearing loss and found that four had mumps and two others probably had mumps. None of their patients had parotitis and each clearly recalled a previous childhood mumps infection.

A large number of viruses of several morphologically different groups are known to cause illnesses of the upper respiratory tract (Mufson et al. 1966) Although most of these viruses are associated with benign disease they are capable of extremely virulent attack upon the central nervous system.

Lindsay (1959) found unilateral sudden deafness, tinnitus and vertigo in four patients who had acute upper respiratory infection with all experiencing partial recovery of hearing during the subsequent few months. Heller & Lindenberg (1955) reported five cases and Lieberman (1957) four patients who experienced sudden deafness with upper respiratory infections.

Some support for a viral etiology for our cases of sudden deafness is found in the high incidence of acute respiratory disease in their clinical histories. At the time of onset of sudden deafness two reported having head-colds, one had acute pharyngitis, and two had pneumonia. Furthermore two of the patients complained of headache suggestive of an associated viral encephalitis.

The similarity of the pathological changes in the eight ears of individuals with sudden deafness to those occurring in ears with known viral labyrinthitis provides further support for a viral etiology Lindsay et al. (1960) reported the pathological findings in the ears of a 6-year-old child who suffered bilateral profound deafness from mumps at the age of 28 months. They found atrophy of the organ of Corti stria vascularis and tectorial membrane. In the basal turns, the organ of Corti was missing whereas in the apical regions supporting elements as well as hair cells were present. The tectorial membrane was severely atrophied in boths ears, appearing either shrunken or transformed into a sphere and encapsulated by a single layer of flat cells. There was a slight loss of cochlear neurons in the basal turns. The vestibular sense organs appeared normal.

Lindsay & Hemenway (1954) described the inner ear pathology in an infant who devel-

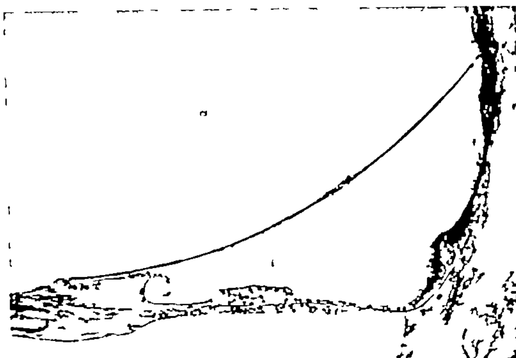


Fig. 21. Ear 8. Case O.S. Cochlear duct in the middle turn of the left cochlea showing the pillar cells and Deiter's cells to be missing whereas hair cells can be identified. The tectorial membrane is displaced into the inner sulcus, and has undergone spherical distortion and partial cellular encapsulation. About 60% of the stria vascularis is degenerated.



Fig. 22. Ear 8. Case O.S. Mid-modiolar section of the cochlea of the left ear showing severe atrophy of the stria vascularis and severe loss of cochlear neurons in all three turns. The loss of cochlear neurons was of equal severity in the opposite ear.

oped measles at the age of 3 months and died of complications of the disease 4 months later Both ears showed atrophy of the organs of Corti which was most severe in the basal turns. The tectorial membranes were degenerated and partially encapsulated. The stria vascularis was atrophied in both ears with the most severe changes occurring in the basal turns. There was a partial loss of cochlear neurons in the basal turn. There was atrophy of the maculae of the utricle and saccule of one ear

Pathological studies of the inner ears of infants suffering from maternal rubella have consistently demonstrated cochleosaccular degeneration (Nager 1952 Lindsay et al. 1953 Hemenway et al. 1969). The changes in maternal rubella were found to be similar to those described for measles and mumps, including atrophy of the organ of Corti, stria vascularis and tectorial membrane. Typically the saccular wall was collapsed onto a partly degenerated otolithic membrane and sensory epithelium The utricles and cristae were normal in these cases.

Beal et al. (1967) reported the pathological findings in the temporal bone of a patient ho experienced sudden profound unilateral ıring loss while suffering from a "head- d They found severe distortion and degeneration of the organ of Corti and stria vascularis with atrophy and encapsulation of the tectorial membrane. In our clinical experience we have occasionally observed a loss of vestibular function in association with measles labyrinthitis but not with mumps or rubella. This tendency to involve predominantly the cochlea is also exhibited in the ears of the individuals with sudden deafness.

The large patent cochlear aqueducts found in the ears of two of the seven cases may be of no etiologic significance however one might speculate that a large cochlear aqueduct establishes an intimacy between cerebrospinal fluid (CSF) and the perilymphatic space which predisposes the inner ear to attack by CSF-laden viral particles. Several common virus infections of the upper respiratory trac (adenovirus, Coxsackie) are commonly com plicated by encephalitis, characterized symp tomatically by headache, photophobia, som nolence and nuchal rigidity The possible rela tionship of the large cochlear aqueduct to sud den deafness remains purely speculative a this time.

Regarding the otosclerotic lesions found i the ears of two of the seven individuals, i seems to us that the occurrence is probabl coincidental, because, in neither case did th bony change involve the endosteum of th cochleae. Basing our judgement on both th clinical and histological features of thes cases, it seems to us that viral labyrinthiti is the most probable etiology for the sudde deafness in these cases.

ZUSAMMENFASSUNG

Pathologische Untersuchungen wurden in acht Fel senbeinen durchgeführt sechs von Patienten mit ei seitiger plötzlicher Taubheit und zwei von einem Pa tienten mit beidseitiger nacheinander folgender plötz licher Taubheit. Der Gehörverlust war tief in vie Ohren, hochgradig in drei Ohren und mittelgradig i einem Ohr Zum Zeitpunkt der plötzlichen Taubheit litten zwei Patienten an Lungenentzündung und zwei klagten über Kopfschmerzen. Schwindel als zusätz liche Erscheinung war schwer in einem Fall und leicht in zwei weiteren Fällen. Die wichtigsten pathologischen Veränderungen bestanden aus Atrophie — in unter schiedlicher Zusammensetzung und Schwere — des Cortischen Organs, der Membrana tectoria und der Stria vascularis. Es wurde beurteilt dass diese patho logischen Befunde denen ähnlicher waren die bei Labyrinthitis mit bekannter viraler Ätiologie auftre ten, als jenen die bei experimentellen Gefässläsionen in Tieren verursacht werden.

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Acta Otolaryng 76 98–108, 1973

ON THE ORIGIN OF THE AUDITORY AVERAGED EVOKED RESPONSES RECORDED FROM THE SCALP IN THE ANESTHETIZED CAT

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(Received November 23 1972)

...aged evoked responses derived ...hetized cats are regarded as ...nducted evoked potentials ... the auditory pathway ... segment of the cochlear ...ex. On the grounds of ... some problems con... generation of vertex po...

...h...nism of the auditory ... (AER) in man is still obscure. There is a perceptible gap in information concerning the role of the primary receiving areas of the cortex and of the subcortical structures in the generation of these responses. As opposed to the common viewpoint (Davis, 1965) an assumption has been recently put forward relating the origin of these responses in the auditory receiving areas of the cortex rather than in the associative areas (Vaughan & Ritter 1970). It is an idea of long-standing (Roth et al., 1956) that the electrical deflections recorded from the scalp in response to sound stimuli (the so-called K-complex) might express the activity of subcortical structures, and it was suggested (Khechinashvili et al., 1972) that during cochlear audiometry the AER of subcortical formations could also be recorded.

Animal experiments will contribute greatly to the solution of problems concerning the mechanism of generation of the human auditory AER. For this purpose, studies were carried out on both extra- and intracra... derived auditory AER in unanesthetized ... guinea pigs and monkeys (Prichard et al. 1965 Kern et al. 1969 a b Hardin & ... lucci, 1970), records and estimates were obtained for the thresholds of AER derived from the dural projections of the auditory cortical areas (Hattory et al., 1971); comparisons were made of the AER of auditory and ... tory cortical areas in anesthetized cats (... sia, 1968).

The object of our experiments was to ... the auditory AER derived from the scalp ... anesthetized cats. The barbiturate ... used in our studies precludes the ... of the AER components caused by the ... contractions and by the activity of the ... ciative system. The simple character of ... responses enables a better understanding ... the components depending on the activity ... the specific auditory structures.

METHOD

Experiments were carried out on 36 ... thetized cats (intraperitoneal injection ... Nembutal, 40 mg kg). The animals were fix... in a stand and placed into a sound-pr... chamber with electrical screening. The AE... were derived from various areas of the ...

ılp (from the vertex in most cases) by means silver disk electrodes of 5 mm diameter ated with electrode paste. Simultaneously AER were recorded from different levels the specific auditory pathway the cochlea, cochlear nuclei the inferior colliculi and auditory cortex. The derivation technique r the evoked responses (ER) of the cochlea d of the subcortical nuclei has been re rted elsewhere (Kevanishvili & Gvacharia, 72) The AER of the auditory cortex were rived from the pial surface with the help ball-tip silver electrodes of 2 mm diameter ıe AER from the scalp and from the cochlea ere derived monopolarly and bipolarly from e subcortical nuclei and the auditory cortex. uring monopolar recordings the reference ectrode was inserted into the neck muscles stainless steel needle) or in the mouth (a ip) in some experiments the reference elec ode was grounded.

The AER of the peripheral and central arts of the auditory system were recorded ı response to clicks and tone bursts. Clicks ere generated by means of 0 05 msec square ulses from an ESU-1 or MSE-40 (Nihon ohden) stimulator applied to a telephone for r earing aids or to a TD-6 dynamic telephone. one bursts of 300 msec duration with a radual rise and fall (8 msec each) were gener ted by a G3–18 oscillator containing in its ırcuit an electronic switch. In most cases ıonaural sound stimulation was realized by neans of elastic ear inlets.

The intensity of tone bursts was measured n dB (re $2\times10^{-5}N/m^2$) by means of the Bruel & Kjaer acoustic measuring instruments. The intensity of click stimuli was neasured in dB peak equivalent of the SPL ry way of comparison (on the screen of an oscilloscope) of their main peak amplitude vith the amplitude of calibrated tonal stimuli.

For the study of somato-sensory scalp-re orded AER the skin of the paw was stimu ated by 0.5 msec single-shock square pulses, he pulses were derived from the stimulator s adiofrequency output.

The ER were averaged by two computers— Didac-4000 (Comef Intertechnique) and Anops-1 (Politechnical Institute of Warsaw). The AVH-2 preamplifiers of the VC 7 oscilloscope (Nihon Kohden) and amplifiers of the MB-5302 electromyograph (Medicor) were used to amplify the derived evoked activity and to observe it on the oscilloscope s screen. The upper limit of the recording system s band-pass could be set in the range of 0 05–15 kHz, the lower limit being 0.5 or 2 Hz. The AER were registered from the oscilloscope s screen of the computers with the help of photographic camera attachments.

RESULT

The AER derived from the scalp in response to clicks generally contain 5–8 negative fast, and 2 slow deflections—a positive and a negative one (Fig. 1 A1) The two initial fast de flections (FD1 and FD2) precede the first slow deflection (SD1), while the remaining (FD3–8) are seen on its descending knee. In response to tone bursts only the SD are re corded (Fig. 1 A2). The latter as well as other features, such as polarity and duration indicates the axon origin of the FD In re sponse to high-intensity sound stimuli in most cases an additional negative hump or plateau occurs before the SD1 (Fig. 1 A3). The latent period (LP) of the FD1 arising in response to clicks applied by insert telephones is equal to 0.8–1 0 msec, whereas that of the SD1 is 2–3 msec. The LP values of the SD peaks depend on the intensity and frequency of sound stimuli (Fig. 2 A) For tone bursts of threshold intensities the LP of the SD1 peak reaches 40–50 msec. As the sound intensity increases, the LP is regularly reduced rarely exceeding 25–30 msec for tone bursts of 90–100 dB. This dependence is less pronounced in respect to the SD2 because of the latter s instability especially in response to tone bursts. With tone bursts of various fre quencies, the smallest peak LP is found with the AER occurring in response to tone bursts

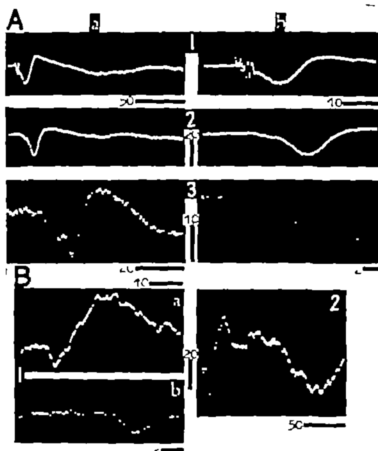


Fig. 1. Auditory (A) and somato-sensory (B) AER recorded from the scalp in the anesthetized cat. A, AER recorded in response to clicks (*1*, *3*) and 2 kHz tone burst (*2*), sound intensity 115 (*1*), 55 (*2*) and 130 (*3*) dB. B, AER recorded in response to skin electroshock stimulation (6 V, 0.5 msec). Frequency of stimulation 1/sec; averaging number, *n* = 100 (A) and 64 (B); band-pass, 15 (A) and 5 (B) kHz. The records on *b* here and in following figures are similar to those on *a* with great expansion of the sweep; negativity is upward, amplitude calibration in μV, time calibration in msec.

of 1 and 2 kHz. For higher frequencies (4 and kHz) the LP are generally 5–10 msec longer. The shortest peak LP are observed with clicks. In addition, as the click intensity changes the LP change, but much less than the LP of the corresponding deflections recorded for tone bursts (Fig. 2 A).

The duration of the initial FD generally does not exceed 0.8–1.0 msec, while that of the later FD is greater. The total duration of AER varies in a wide range. Of 300 responses picked out at random among the records of 15 experiments the duration of 255 responses (85%) did not exceed 130 msec. In the most of cases it was in the range of 80–120 msec with deviation from 50 to 200 msec.

The amplitude of the AER from the SD1 peak to the SD2 peak with threshold intensities is 1–5 μV. The amplitude grows with the stimulus intensity, reaching generally 20–30 μV for intensities of 90–100 dB (Fig. 2 B).

The FD are several times lower than the SD. As a rule, the highest amplitude is typical for the FD4 and FD3, while the lowest one for the later deflections (FD5–8); the latter are not always pronounced (Fig. 1 A3, 6A).

The amplitude of the SD recorded in response to monaural stimulation are practically the same when being recorded from the vertex and from the contra- and ipsilateral areas of the scalp. This can be said also about the FD3 and FD4 and the later spikes. However the ipsilateral FD1 and FD2 are sometimes slightly greater than the contralateral ones.

The thresholds of the AER recorded in response to tone bursts are within the range of 33–54 dB (Fig. 3, *I*). For the frequencies of 1 and 2 kHz the thresholds are lower than for higher (4 and 6 kHz) and lower (0.25 and 0.5 kHz) ones. The highest threshold is observed for clicks. In a given animal the threshold varies within the range of ±5–10 dB (Fig.

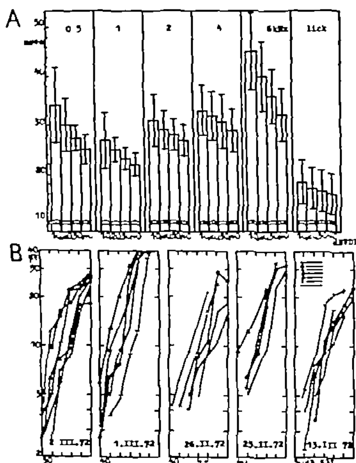


Fig. 2. Dependence of the peak LP (A) and of the amplitude (B) of the auditory AER on the intensity and frequency of sound stimuli. *Abscissa*: intensity of sound stimuli in dB re visual detection level (A) and 2 · 10^{-5} N/m² (B); *ordinate*: average ($n = 11$) and standard deviation LP values of the SD1 peak in msec (A) and amplitude values in μV (B). Marks on B (from top), 0.25, 0.5, 1, 2, 4, 6 kHz and click.

3: 2, 3). With various effects on the sound-conducting and sound-receiving elements such as sound trauma (Fig. 3: 4–7), antibiotic insults and various surgical interventions on the middle and inner ear, the thresholds increase in correspondence to the degree of the disturbance. In the course of time the thresholds undergo corresponding shifts.

Similarly to other AP of fibre tracts the FD are characterized by a short recovery cycle. The obvious decrease of their amplitude is observed only with stimulation rates exceeding 20/sec, while the SD can follow without *decrement only to low rhythms.* The reduction of the latters starts at stimulation rates exceeding 2/sec, though they never disappear completely, especially the SD1, even at considerably higher stimulation frequencies. As opposed to rhythmic stimulation with paired clicks *the SD amplitudes arising on test stimuli* start to reduce with the conditioning intervals of 120–90 msec.

The AER recorded from the vertex in response to the skin electroshock stimulation (Fig. 1 B) differ from the auditory AER by a significantly greater LP and by the absence of FD. Besides, in the somato-sensory AER additional SD are observed.

The comparison of the AER derived from the scalp and that derived from the auditory cortex (Fig. 4 A) reveals significant differences, e.g., the LP of the cortical AER is several times greater than that of the SD1. There are some other differences as well, such as the absence of the FD in the auditory cortex records and different waveform and duration of the SD. *On the other hand, the AER* derived from the scalp change definitely af

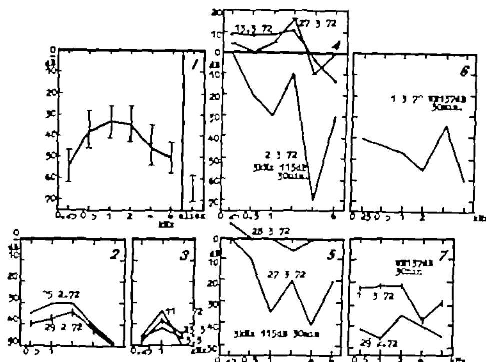


Fig 3. Average ($n = 17$) and standard deviation threshold values of the scalp-derived auditory AER (*1*) threshold variability of the AER (*2 3*) threshold changes of the AER after high-intensity pure-tone (*4 5*) and white-noise (*6 7*) exposures. *Abscissa* frequency of sound stimuli in kHz; *ordinate* thresholds of the AER in dB re 2×10^{-5} N/m² (*1–3*) and n thresholds before the sound trauma (*4–7*).

ter bilateral removal (Fig. 5 B) or freezing of the cortical auditory areas. This indicates a possible participation of the auditory cortex in the formation of the AER derived from the scalp. Besides that a recovery cycle similar to that of the geniculo-cortical system is typical for the SD derived from the scalp.

After the removal of the auditory cortex additional sections of the cerebral white matter under the electrode location do not affect the AER derived from the scalp, while the brain-stem section above the inferior colliculi causes a certain reduction of these responses (Fig. 5 B).

Isolation of the scalp recording area by a circular section of the soft tissues and by separation of the bone causes a certain reduction of the amplitude both of the FD and SD (Fig. 5 A).

The auditory AER derived from the scalp differ from the AER of the inferior colliculi in regards of the LP (the LP of the inferior colliculi's AER is longer) and the duration both of the individual components and of the total response. A difference also exists in the recovery cycle: the inferior colliculi's AER to tested stimuli start to reduce for the conditioning intervals of 40 msec (Fig. 4 B).

The LP of the start of the SD1 derived from the scalp and of the AER of the cochlear nuclei coincide. The LP, the duration and the waveform of some FD are also alike. However in general, there are some remarkable differences between them, such as the difference in the shape and duration of the SD. They strongly differ from one another by the recovery cycle: it is much less for the AER of the cochlear nuclei (Fig. 4 C).

After a brain-stem section on the level or below the inferior colliculi the AER derived from the scalp change strongly, but they never disappear (Fig. 5 C). The SD remaining after such section can follow to high rates of stimulation. This features as well as the

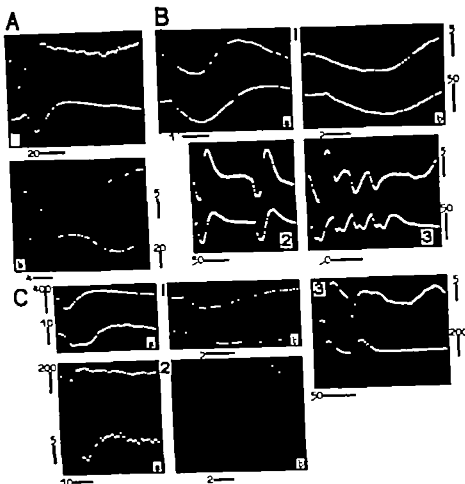


Fig. 4. Comparison of AER derived from the scalp and from the primary auditory cortex (A), the inferior colliculi (B), and the cochlear nuclei (C). *1st sweep:* the AER recorded from the scalp (A, B, C3) and from the cochlear nuclei (C1, 2); *2nd sweep:* the AER recorded from the auditory cortex (A), the inferior colliculi (B), the cochlear nuclei (C3) and the scalp (C1, 2). Repetition frequency 1 sec. On 2 and 3 stimulation with 2 and 4 clicks respectively: interval between the clicks, 120 (B2), 30 (B3) and 50 (C3) msec: click intensity 100 (A, C) and 90 (B) dB. $n = 64$. band-pass, 5 (A, C2) and 0.05 (B, C1, 3) kHz. On A and C2 the FD waveform is not complete due to the large bit width (0.4 and 0.2 msec respectively); on B and C1, 3 the FD are absent due to the low-frequency band-pass.

total duration and shape makes the remained deflections resemble the auditory AER of the rhombencephalic level.

In many characteristics (LP occurrence in response to short stimuli, equal polarity duration and recovery cycle) the FD1 and FD2 of the scalp-recorded AER are similar to the cochlear N and N potentials, respectively (Fig. 6 A). However, together with the other FD and SD the FD1 and FD2 disappear after sectioning of the 8th nerve or after application of Novokain to its intracranial segment, which do not affect significantly the cochlear electrical activity (Fig. 7). The FD1 and FD2 differ from the cochlear nervous potentials also in amplitude ratio: the FD1 is equal to or is less than the FD2, while the N_1 potential is 2–3 times higher than the N_2 potential (Fig. 6 A).

The polarity, the LP and the duration of the SD1 are similar to those of the cochlear SD (Fig. 6 B), recently investigated in detail

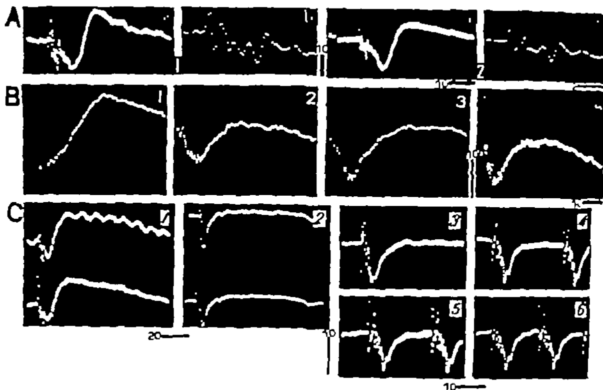


Fig. 5. Effect of isolation of the recording area (A), of removal of the auditory cortex (B2), and of brain stem sections (B3, 4, C) on the scalp-derived AER. A, the scalp-derived AER before (*1*) and after the section of the skin and separation of the bone around the recording area (*2*). B, the AER recorded from the scalp after opening of the auditory cortical areas (*1*), after bilateral removal of the auditory cortex (*2*), after section of the white matter under the derivation area (*3*), and after section of the brain stem above the inferior colliculi (*4*). C, the scalp-derived AER before (*1*) and after section of the brain stem below the inferior colliculi (2–6). Frequency of stimulation, 1 (A, B, *C1–3*), 36 (C4), 45 (C5) and 57 (C6) per sec; click intensity 115 dB; *n* = 64; band-pass, 5 kHz. On *C1, 2* the FD waveform is not complete due to the large bit width (0.4 msec).

or guinea-pigs (Kupperman, 1971). Regardless of these similarities the SD1 can not be considered as a volume conducted SD of the cochlea. First of all, they differ in recovery cycles which are much shorter for the cochlear SD. Second, a section of the 8th nerve does not affect the cochlear potentials, whereas the records from the scalp show a disappearance of AER.

DISCUSSION

The described data indicate that the auditory AER derived from the scalp in anesthetized cats do not express an evoked activity of a certain level of the auditory pathway. Considering all the similarities and differences with the AER of individual levels of the auditory system one can come to the conclusion that they are of a complex origin, formed out as a result of the algebraic summation of ER spreading out by the way of volume conduction from various levels of the system. Principal sources of these AER are the auditory fibres and nuclei of the bulbar, pontal and mesencephalic levels. This is justified, first of all, by the preservation of the FD and SD after transsections of the brain stem above the inferior colliculi and by the short LP of the initial FD and SD1.

Yet before averaging computers came into application many investigators had succeeded in recording ER at significant distances from the sites of their origin (Chang & Kaada,

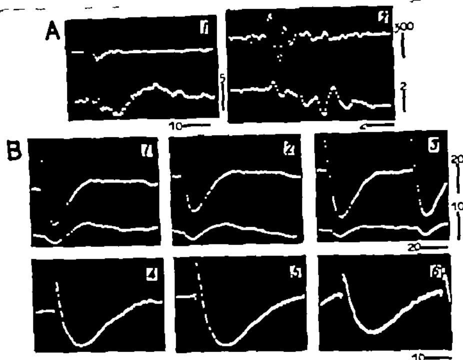


Fig. 4. Comparison of the scalp-derived AER with the cochlear evoked potentials. *1st sweep*: cochlear FD (A) and SD (B); *2nd sweep*: the scalp-derived AER. Frequency of stimulation, 1 (A), 2 (B1, 4), 5 (B2), 10 (B5), 15 (B3) and 25 (B6) per sec; click intensity 105 (A) and 115 (B) dB; $n = 64$ (A, on 2, 32 in condensation and 32 in rarefaction phases) and 32 (B); band-pass, 5 (A) and 0.05 (B) kHz. On B the FD are absent due to the low-frequency band-pass.

1950; Rosenblith & Rosenzweig, 1951; Grinnell, 1963; Meshcherski, 1966). The computers made this task much easier. Thus, in guinea-pigs (Kern et al., 1969 *b*) and cats (Celesia, 1968) scalp responses were recorded to clicks, which on the basis of the LP values were considered to be the ER of the cochlear nuclei and of the inferior colliculi (Kern et al., 1969 *b*) and of the medial geniculate body (Celesia, 1968). It was presumed (Chang & Kaada, 1950) that volume conduction occurs in the brain along the nervous fibres, through the surrounding liquor. Retaining of the AER in the records from the scalp after brainstem sections proves the volume conduction of the ER along the nervous fibres as well as through other conducting media. Conduction of the ER might be realized to some extent even through the scalp bones. The latter agrees with the experimental data of Mikaelian (1967) on volume conduction of the ER of the cochlear nuclei.

As to the general shape, the frequency threshold curve of the studied AER is in close relevance to the auditory threshold curve obtained in behavioural studies (see Hayden, 1965). The frequency selectivity is reflected also in the relative values of the peak LP: they are lower with the application of tone bursts of 1 and 2 kHz, are slightly greater at 0.5 kHz and are significantly greater at 4 and 6 kHz. These differences might be due to different conditions for spatial summation depending on the activation of receptor cells in different spaces of the basilar membrane (see Davis & Silverman, 1960). The comparatively short LP of the SD arising in response to clicks as well as high click thresholds and low sensitivity of the LP to the stimulus intensity changes should be explained by the

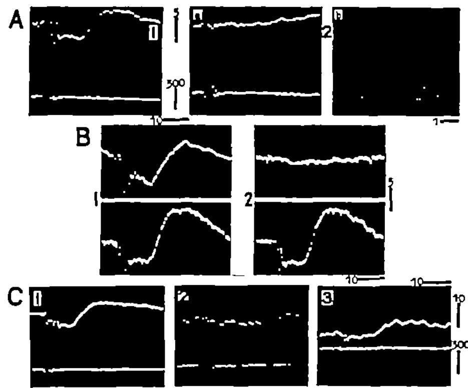


Fig. 7. Effect of section of the 8th nerve (A, B) and application of Novokain to its intracranial segment (C) on the scalp-derived AER (B: 1st sweep on A and C) and on the cochlear potentials (2nd sweep). A, B, AER and cochlear potentials before (*1*) and after the section of the 8th nerve (*2*), with stimulation of the intact ear (bottom records on B) ordinary AER are recorded. C, the AER and the cochlear potential before (*1*), immediately (*2*) and after 2 h (*3*) of application of 0.5% Novokain on the 8th nerve. Frequency of stimulation 1/sec; click intensity 105 dB, $n = 128$; bandpass, 5 kHz.

...ort duration of the click's main peak which ... the efficiency of the temporal summation (Gershuni et al. 1964, Rapin 1965).

The LP and other features of the FD1 are similar to the cochlear N_1 potential. Therefore the FD1, similarly to the N_1, may be considered as a compound AP of the 8th nerve. The difference between them seems to lie only in the site of origin. The nervous potentials recorded from the round window or from the cochlea are known to express the excitation of intracochlear fibres, while the FD1 seems to be of retrocochlear origin. This is suggested by the disappearance of FD1 as well as of all other deflections after the intracranial section of the 8th nerve or after application of Novokain. Assuming the FD1 as the N_1 to be the AP of the 8th nerve, we could consider the mechanism of generation of the FD2 similarly to the N_2 potential. But this is denied out by the amplitude correlation between the FD1 and FD2, which is different from the correlation between the N_1 and N_2 (see Salomon & Elberling, 1971). In addition, the threshold of the N_2 potential is higher than the one of the N_1 (Kupperman, 1971), while the thresholds of the FD1 and FD2 are equal. Proceeding from the above one could presume that the FD2 expresses the excitation of the central rather than of the peripheral acoustic fibre bundles.

Hence it can be suggested that the short-latency FD of equal amplitudes recorded at some distances from the cochlea in animals (Rosenblith & Rosenzweig, 1951, Grinnell, 1963) and in man (Sohmer & Feinmesser, 1967, Keidel & Spreng, 1970) do not express the excitation of the intracochlear fibres. The

first AP should express the excitation of the retrocochlear 8th nerve fibres while the second one—the excitation of a bundle of intracerebrally located acoustic fibres. In the latter case one should imply also a possible role of the 8th nerve's "slow" retrocochlear fibres.

The presented data conform and, possibly supplement the recently published material (Jewett, 1970; Lev & Sohmer, 1972) on similar FD led off from various extracochlear sites (n. caudatus, pinna etc). Consistent with this material the later FD can be regarded as the AP of following order auditory fibres. Considering the whole complexity of afferents of the specific auditory nuclei there is every reason to accept the viewpoint (Jewett, 1970) that individual FD must be related to various auditory nuclei rather than to a single one, though the relation with one of them may be dominant.

It was suggested (Khechinashvili et al., 1972) that the FD with great LP recorded during cochleography in man express the evoked activity of the central, but not of peripheral formations of the auditory tract. The data presented now on the possibility of recording from different scalp areas not only of the AP of the 8th nerve but also of the bulbar, pontal and mesencephalic acoustic fibres supports this suggestion. As is shown by our experiments, the polarity and the duration of the AP of the central acoustic fibres recorded from different scalp areas do not differ from that of the 8th nerve, while the amplitude of the formers are very often higher and are better observed at threshold intensities of sound stimuli. Therefore, these potentials might be easily confused if the LP values are not taken into account.

An assumption was made (Davis et al., 1966; Heath & Galbraith, 1966; Khechinashvili et al., 1972) on the complex nature of the auditory AER derived in man from the vertex. It was indicated (Khechinashvili et al., 1972) that these AER might reveal the phenomenon of circulation (reverberation) of excitation in many complex circuits, such as, between the cortex and subcortical structures, the associative and the primary cortical areas, etc. Our results on the complex origin of the AER recorded in anesthetized cats under similar recording conditions support this view. Proceeding from these results and keeping in mind the known conditions (e.g. the localization of electrodes) optimal for the derivation of relatively high-amplitude human AER (Rapin, 1965; Celesia et al., 1968) one could suggest that a certain role in the generation of these responses is played by the midline cerebral structures, namely the cingular gyrus and other supra- and infracallosal formations. Following these assumptions the known differences between the AER derived from the human scalp and from the cerebral cortex (Heath & Galbraith, 1966; Celesia et al., 1968) could be related to the subcortical volume conducted components summated with the cortical ER.

And, finally, it should be mentioned that the study of the auditory AER derived from the scalp in anesthetized cats seems to be important from the methodological point of view: they might provide a tool for sufficiently accurate audiometric investigation in animals.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Professor S. N. Khechinashvili for guidance during this work and valuable suggestions.

ZUSAMMENFASSUNG

Von der Kopfhaut narkotisierter Katzen wurden durch Schnalz- und Krachlaute hervorgerufene Potentiale abgeleitet. Für den Komplex der schnellen und langsamen elektrischen Reizantworten werden die Charakteristiken angegeben. Die erhaltenen Daten deuten darauf hin, dass dieser Komplex das Ergebnis einer algebraischen Summation der nichtsynaptisch weiter geleiteten evozierten Potentiale ist, die in verschiedenen Stufen der spezifischen Hörbahn — N. cochlearis bis Hörzentrum — entstehen. Unter Berücksichtigung der vorliegenden Ergebnisse werden einige Probleme des Entstehungsmechanismus von ermittelten, akustisch evozierten Potentialen, wie sie beim Menschen erhalten werden, diskutiert.

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Acta Otolaryng 76 109–113 1973

AUDITORY AGNOSIA DUE TO INCISION OF SPLENIUM CORPORIS CALLOSI

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(Received August 5 1972)

Abstract. The symptoms in a case of auditory agnosia are described. The patient, a 13-year-old boy underwent surgical operation for pinealoma, in which the splenium of corpus callosum was incised. After the operation he suffered from auditory agnosia. There were pure word-deafness and musical agnosia. The inner language was intact and the ability to read and write was unimpaired. Standard pure tone audiometry revealed a slight bilateral hearing loss at the low frequencies. With the speech audiogram, the verbal perception was poor in both ears, especially the left. On the other hand the musical test showed the melody perception was poorer in the right ear Within 2 months of the episode, the patient recovered from auditory agnosia to normal limits.

Auditory agnosia is usually associated with word blindness, paraphasia and agraphia, which together make up the picture of Wernicke's aphasia (Wohlfart et al. 1952). Oohashi (1967) classified auditory agnosia in psychic deafness, sensory amusia and pure word-deafness.

We have had the opportunity of observing a patient in whom there was auditory agnosia (verbal and musical) but no word blindness, paraphasia or agraphia. His inner language was intact. It is the purpose of this paper to report the clinical observations in this case and to discuss these in relation to the central auditory function.

CASE REPORT

Patient 13-year-old boy (1972).

History In August 1971 there was a gradual onset of a tremor in the right hand, followed by difficulty in writing. Two months later he felt a numbness and weakness in the right arm and leg. He was admitted to the hospital for a check-up in November 1971 His physical condition was within normal limits. An otological examination revealed no abnormality An electroencephalography was almost normal. The pinealoma was suspected at neurological examination and X-ray including angiography and pneumo-encephalography As treatment, radiation therapy with Co^{60} (3 000 R) was performed for 20 days and the tumor was then removed surgically in February 1972.

At the cranial operation, the splenium corporis callosi was incised about 2 cm in sagittal axis to attack the tumor Other parts of the brain were not involved at all. Within a few days of the operation, his complaints, including tremor and ataxia, were healed. However the day after the operation the patient noted an inability to understand spoken words, although he heard sounds. His consciousness was clear and his inner language seemed to be unimpaired.

Examination. The examination was done three times, 7 days, 30 days, and 60 days after the episode of auditory agnosia. The neurological examination except for an auditory agnosia was within normal limits. The IQ measured by the nonverbal subtest of the Wechsler Intelligence Scale for Children, was 90. His intelligence was considered as of a normal range.

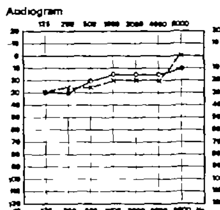


Fig. 1 Pure tone audiogram. Air conduction was tested on the 7th day after the episode. o—o, right ear ×---× left ear

(A) *Hearing tests*

(1) *Standard pure tone audiometry* As shown in Fig. 1 the audiogram showed a slightly diminished hearing in the lower frequency range.

(2) *Speech audiometry* Within a week of the operation, the speech audiogram (Fig. 2) revealed that the patient had difficulty in the discrimination of monosyllabic words, especially in the left ear However the discriminating ability improved remarkably in a month, as shown in Fig. 3

) *Examination of the aphasia and agnosia*

The patient was right-handed and had no disturbance in the inner language and articulation.

(1) *Voluntary speech.* The voluntary speech was practically unimpaired. He had neither paraphasia nor agramatism His speech was correctly formed with a normal rhythm and his vocabulary was not diminished. There was no tendency to word perseveration.

(2) *Auditory comprehension* The result of the test in 7 days after the episode was as follows; He could not understand the spoken words, but was able to lip-read sometimes. He could not recognize his own name when spoken. Spoken words and other sounds were indistinguishable. But he could discriminate occasionally between certain everyday sounds, such as the noise made by a door or telephone bell. Within a month he was able to understand some spoken words. Moreover in 2 months he had almost no difficulty in easy conversation.

(3) *Melody recognition* The patient was asked to identify melodies delivered to each ear separately The test materials were familiar to him. The result is shown in Fig. 4 The score for the left ear was within the normal range However the score for the right ear was poor on the 7th day after the episode It was found that with the right ear he suffered from defective interpretation of musical sounds and defective musical memory However his faculty of melody recognition in the right ear improved remarkably to normal limits within 2 months.

(4) *Reading* He could read silently and aloud without difficulty There was no disturbance of intonation and pause. He was able to comprehend correctly the content of what he read.

(5) *Writing* He was able to write, and copy typewritten texts. He made no mistakes in his writing and spelling.

After the episode of auditory agnosia, contact with patient was achieved by writing usually But he was able to hear certain simple words when his attention was focused for lip-reading. So it was arranged for him to take lip-reading and auditory training lessons in his family every day The auditory agnosia

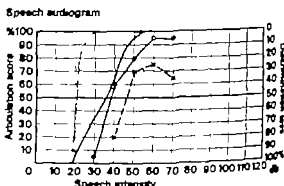


Fig 2 Speech audiogram. This test was carried out on the 7th day after the episode. o—o, right ear ×--- left ear

improved gradually and in a month he was able to understand simple words when spoken clearly and slowly In 2 months his progress was so pronounced that he could understand other people in ordinary conversation without difficulty In 2 months after the episode the examination showed normal limits as regards speech audiogram and a melody test. He recovered almost completely from auditory agnosia in 3 months.

DISCUSSION

The case presented here had an auditory agnosia including verbal agnosia and sensory amusia. Similar cases have been reported earlier as "pure word deafness" So-called pure word deafness is extremely rare Pure word deafness is classified as a speech disorder in which the ability to understand spoken language, repeat words and write from dictation is lost, while the ability to speak, write and read spontaneously is preserved (Jones & Dinolt, 1952). Our patient was able to hear but could not understand what was heard, after the splenium corporis callosi was incised surgically He had a verbal and musical agnosia without word blindness and paraphasia. His inner language was intact. Tanaka et al. (1964) reported pure auditory agnosia associated with alteration of voice and personal character Wohlfart et al, (1952) reported the pure word deafness, in which post

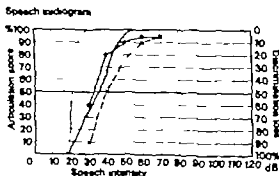


Fig. 3 Speech audiogram. This test was done on the *30th day after the episode.* o—o *right ear* - - *left ear*

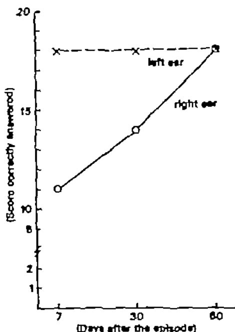


Fig. 4 The melody-recognizing test was carried out on right and left ear separately

mortem investigation revealed the coexistent changes of both temporal lobes. On the other hand, cases with a single subcortical lesion in the left temporal lobe have been described (Schuster & Taterka, 1926) Nielsen (1962) stated that "there are two levels of integration primary perception and eugnosia. Destruction of the first results in deafness, of the second in agnosia—acoustic or auditory agnosia if complete, acoustic verbal agnosia if recognition of words only is lost" He was of the opinion that Wernicke's region is considered with the entire function of auditory recognition. That is, the lesion causing pure verbal agnosia must interrupt fibers from both primary auditory areas to Wernicke's center on the major side. Morel (1935) considered that psychic deafness always accompanies either sensory amusia or word deafness, or both.

Each ear has connections with the auditory receiving area in each hemisphere, but the pathways connecting the ears to their opposite hemispheres are apparently more effective than the ipsilateral pathways (Rosen-

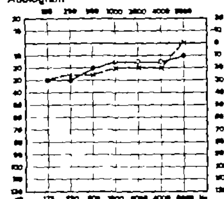


Fig 1 Pure tone audiogram. Air conduction was tested on the 7th day after the episode. o—o right ear ×---× left ear

(A) *Hearing tests*

(1) *Standard pure tone audiometry* As shown in Fig. 1 the audiogram showed a slightly diminished hearing in the lower frequency range.

(2) *Speech audiometry* Within a week of the operation the speech audiogram (Fig. 2) revealed that the patient had difficulty in the discrimination of monosyllabic words, especially in the left ear However the discriminating ability improved remarkably in month as shown in Fig. 3

) *Examination of the aphasia and agnosia* The patient was right handed and had no disturbance in the inner language and articulation.

(1) *Voluntary speech* The voluntary speech was practically unimpaired. He had neither paraphasia nor agramatism His speech was correctly formed with a normal rhythm and his vocabulary was not diminished. There was no tendency to word perseveration.

(2) *Auditory comprehension.* The result of the test in 7 days after the episode was as follows He could not understand the spoken words, but was able to lip-read sometimes. He could not recognize his own name when spoken. Spoken words and other sounds were indistinguishable. But he could discriminate occasionally between certain everyday sounds, such as the noise made by a door or telephone bell. Within a month he was able to understand some spoken words. Moreover in 2 months he had almost no difficulty in easy conversation.

(3) *Melody recognition* The patient was asked to identify melodies delivered to each ear separately The test materials were familiar to him. The result is shown in Fig. 4 The score for the left ear was within the normal range However the score for the right ear was poor on the 7th day after the episode. It was found that with the right ear he suffered from defective interpretation of musical sounds and defective musical memory However his faculty of melody recognition in the right ear improved remarkably to normal limits within 2 months.

(4) *Reading* He could read silently and aloud without difficulty There was no disturbance of intonation and pause He was able to comprehend correctly the content of what he read.

(5) *Writing* He was able to write, and copy typewritten texts. He made no mistakes in his writing and spelling.

After the episode of auditory agnosia, contact with patient was achieved by writing usually But he was able to hear certain simple words when his attention was focused for lip-reading. So it was arranged for him to take lip-reading and auditory training lessons in his family every day The auditory agnosia

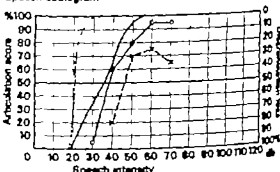


Fig 2. Speech audiogram. This test was carried out on the 7th day after the episode. o—o right ear ×---× left ear

stressed that at least until the age of about ten to twelve years, extensive recovery is expected, usually within weeks or months, even after hemispherectomy. Our patient was 13 years old. The brain lesion was only a small part of the splenium corporis callosi and other parts were intact. Thus auditory agnosia was remarkably improved within a few months.

ZUSAMMENFASSUNG

Ein Fall von akustischer Agnosie wird geschildert. Ein 13 Jahre alter Knabe litt am Pinealom, und dieser Tumor wurde chirurgisch exstirpiert. Während der chirurgischen Operation wurde die Splenium corporis callosi etwa 2 cm sagittal geöffnet. Nach der Operation litt der Kranke an akustischer Agnosie und sensorischer Amusie. Er verstand die gesprochene Sprache nicht. Die Fähigkeit zu lesen und zu schreiben war nicht beeinträchtigt. Das spontane Sprechen und die geistige Sprache waren normal. Ein leichter binauraler Hörverlust für tiefe Frequenzen wurde beobachtet. Nach der Sprach-Audiometrie bei monauraler Darbietung zeigte sich die Verständnisfähigkeit der beiden Ohren, besonders des linken Ohres, geschädigt. Aber bei musikalischer Prüfung fand sich das Verstehen von Melodien beim rechten Ohr stärker beeinträchtigt. Zwei Monate nach dem Anfall war der Kranke von der akustischen Agnosie wieder geheilt.

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Acta Otolaryng 76 114–120 1973

DIRECTIONAL AUDIOMETRY

VIII *The Influence of Hearing Aid on the Localization of White Noise*

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(Received February 1973)

Abstract. The ability to localize white noise in the horizontal plane without and with hearing aids in an anechoic room was investigated in 80 patients. It was found that impaired hearing did not necessarily entail poor directional hearing. Asymmetrical hearing loss seemed to have a more pronounced effect on the directional hearing than approximately symmetrical hearing loss. Head-borne hearing aids of the type used here cannot be expected to improve directional hearing whilst, in patients with approximately symmetrically impaired hearing, directional hearing was less disturbed by binaural than by monaural aids. Directional hearing can be expected to deteriorate with increasing age. The patients own impression of their directional hearing did not always agree with our results. The conditions described have only validity for our patients as a group and do not allow any conclusions to be made about the individual patient's directional hearing, which must be evaluated individually

rmal hearing with pure tone audiometry s not necessarily mean that directional earing is normal just as impaired hearing is not always accompanied by poor directional hearing. Even monaural deafness does not prevent the perception of direction (e.g. Fisher & Freedman, 1968) The lack of correlation between the pure tone audiogram and directional hearing is illustrated by the fact that patients with otosclerosis were found to have poor directional hearing whether they had been operated (fenestration) or not (Jongkees & van der Veer 1957 van der Veer 1957).

The evaluation of directional hearing in a patient using a hearing aid is complicated A hearing aid usually prevents the auricles from functioning. Several researchers consider that the auricles are of significance for directional hearing (Batteau, 1967 Fisher & Freedman, 1968) The work reported here was undertaken with the object of investigating directional hearing for white noise in the horizontal plane in patients with different types of impaired hearing

APPARATUS

The apparatus used to measure the directional hearing ability for the twelve positions of a white noise source in the horizontal plane was the same as described in a previous publication (Tonning 1970) The stimulus used was white noise of 65 dB re. 0.0002 dyne per square centimetre measured 0.9 metre from the signal loudspeaker without any person in the sound field. This point corresponded to the centre of the head of the person examined

METHOD AND MATERIAL

The ability to localize white noise in the horizontal plane without and with hearing aids was examined in 80 patients with different types of hearing loss. The method used for this examination has been described earlier (Tonning 1970) The hearing loss is given as PTA i.e. Pure Tone Average the mean of the hearing levels at the frequencies 500, 1 000, and 2 000 Hz. The audiometer was calibrated according to British Standard 2497 1954 The relationship between British Standard 1954 and ISO Standard 1964 for these frequencies is shown in Table I (see also Whittle & Delany 1966)

Table 1 *The relationship between British Standard 1954 and ISO Standard 1964 for our equipment (earphone THD 39/MX41AR, 9A coupler)*

The table indicates the number of dB that must be added to the threshold of hearing recorded in British Standard when transferring to ISO Standard

Frequency Hz	00	1 000	2 000
dB added to hearing loss in British Standard when transferring to ISO Standard	+2.0	+2.9	−0.2

Investigations were made without and with hearing aids. The 80 patients examined were divided into 4 groups:

Group A. 20 patients with monaural hearing loss varying from a PTA of 30 dB hearing level to residual hearing (residual hearing: hearing measurable at only some of the frequencies tested). These 20 patients and the hearing aids used on the bad ear are described by Tonning (1972 *a*).

Group B. 20 patients with monaural residual hearing or no measurable hearing at all in one ear. The patients and hearing aids used are described by Tonning (1972 *b*). The microphone of the hearing aid was placed near the meatus acusticus externus of the bad ear and the receiver delivered the signal to the good ear by means of a polyethylene tube that did not occlude the ear canal. In the literature this form of hearing-aid-treatment is often designated "Contralateral Routing of Signals" CROS.

Group C. 20 patients with no demonstrable hearing in one ear and a PTA between 23 and 55 dB hearing level in the other ear. These patients and the hearing glasses used are described by Tonning (1972 *c*). There was a microphone in each templepiece. These two microphones had different frequency responses. The impulses from both microphones were transmitted to an amplifier in the templepiece at the best ear. From a telephone in the same templepiece the sound was transmitted via a tube to the best ear. Five patients did not use ear-moulds.

Group D: 20 patients with binaural hearing loss. The PTA was between 28 and 45 dB hearing level. The greatest difference between the PTA of the two ears in any one patient was 7 dB. These patients and the hearing aids used are described by Tonning (1973). The patients were examined without hearing aid, with monaural and with binaural aids.

Bauer et al. (1966) found that "reorientation in azimuth localization with earplugs inserted required three days or more unless accelerated by specific training". For this reason the patients were first examined without hearing aids. Aids had been used for at least two months before the tests with hearing aids were made. The patients in Group D were examined with monaural and binaural aids in two seances after an adaption interval of at least one week.

The patients were not told if their statements were correct. Perrott & Elfner (1968) and Elfner et al. (1970) have shown that information of this kind improved the reports. Angell & Fite (1901) recorded that much repetition of the measurements improved the reports somewhat even when the subjects were not told if their statements were correct. Only 12 localizations were made by our patients at each session. One person with normal hearing and three with impaired hearing each made 24 consecutive localizations. No significant difference was found in the tendency of localization between the first 12 determinations of direction and the last 12 (Wilcoxon Two Sample Test, level of significance 0.05).

Thus we decided to assume that the investigation itself did not affect the patients ability to localize the noise source.

RESULT

(a) The directional hearing ability of the patients with and without hearing aids as compared with 30 normally-hearing persons

An investigation was made of the degree to which our patients both with and without hearing aids localized the loudspeaker as compared with 30 normally hearing standard sub-

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Normal hearing with pure tone audiometry does not necessarily mean that directional hearing is normal, just as impaired hearing is not always accompanied by poor directional hearing. *Even monaural deafness does not* prevent the perception of direction (e.g. Fisher & Freedman, 1968) The lack of correlation between the pure tone audrogram and directional hearing is illustrated by the fact that patients with otosclerosis were found to have poor directional hearing whether they had been operated (fenestration) or not (Jongkees & van der Veer 1957 van der Veer 1957)

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Group C. Five patients localized the source of noise towards the side of the best ear. For an additional patient this was more pronounced with a hearing aid with binaural microphones. Without a hearing aid another patient localized chaotically whilst with a hearing aid he stated consistently that the sound was opposite the best ear. Yet another patient showed the inverted tendency without a hearing aid the source of noise was localized opposite the best ear whilst treatment with a hearing aid resulted in apparently random reports. The material did not allow any close examination of the influence of earplugs on the determination of direction.

Group D. In three patients with monaural hearing aid the source of noise was localized to the side wearing the apparatus, as described by Halbrock et al. (1959). This localization was not, however, especially pronounced in our patients and no systematic lateralisation could be detected either without or with two hearing aids.

Division of the patients into groups, as in Fig. 1 showed that asymmetrical loss of hearing seems to have a marked effect on directional hearing regardless of whether the other ear has impaired hearing or not; this applies both with and without hearing aid.

The relation between the degree of one-sided impaired hearing and the ability to localize the source of noise without a hearing aid has been investigated for groups A and B. The Spearman Rank Correlation Coefficient was found to be significantly greater than 0 (C = 0.687). A connection between the degree of one-sided hearing loss and the ability to localize the source of noise is therefore assumed: the poorer the PTA the poorer the ability to localize. Viehweg & Campbell (1960) had the same experience.

Our material does not permit an evaluation of the directional hearing of the individual patient on the basis of the type, degree and duration of the hearing loss. Van der Veer (1957) made a corresponding report as regards the type of hearing loss. Viehweg & Campbell (1960) had the impression that patients with conductive hearing loss localized best and those with sensorineural hearing loss worst, but this could not be tested statistically. The authors did not find connection between the duration of the loss of hearing and the directional hearing.

(b) The effect of hearing aid on the directional hearing ability

It is evident from Fig. 2 that, in our experimental conditions, treatment by means of hearing aid cannot be expected to lead to significant improvement of directional hearing (Wilcoxon-Test for Paired Comparisons, level of significance 0.05).

Group A. With hearing aid, directional hearing was better for two patients and worse for two patients. Harford & Musket (1964) reported that some of their patients in the same category as Group A themselves had the impression that they could localize noise better with a hearing aid whilst others did not have this impression. Butler & Naunton (1967) examined the directional hearing of 9 normally hearing subjects wearing a muff over one ear. All the listeners displaced the noise towards the unoccluded ear. Further "the apparent location of sounds originating on the side of the occluded ear more closely approximated their actual position when the stimulus intensity was increased". This was considered an expression of the increasing influence of interaural time differences at the higher sensation levels. Seen from this point of view one would expect our patient group A to achieve better directional hearing when monaural hearing aid was used. However Fig. 2 shows no convincing positive effect of hearing aid on directional hearing.

Group B. With the CROS aid only one patient showed improved ability to localize whilst four localized less well. Harford & Barry (1965) reported that 11 out of their 20 patients themselves considered that their ability to localize was improved with CROS. Rintlemann et al. (1970) reported two blind patients with uni-

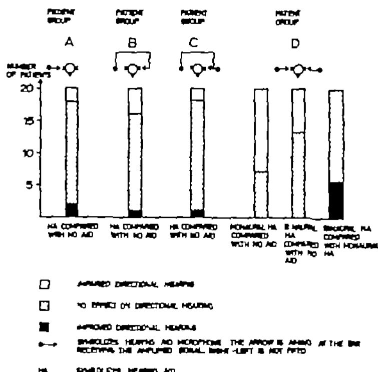


Fig 2 The influence of hearing-aid-treatment on the directional hearing ability

lateral hearing loss where CROS was thought to improve directional hearing

Group C Only one patient had improved directional hearing with hearing aid while two localized less well with hearing aid.

Group D Treatment by means of monaural aring aid led to the result that 13 patients ad poorer directional hearing than without n apparatus. The use of binaural aids reduced this total to seven patients. Directional hearing for five patients was significantly better with binaural than with monaural apparatus. This agrees well with di Carlo & Brown (1960) and Halbrock et al. (1959) The latter found, as we did, that the source of noise was often localized towards the side supplied with the aid and that this poor directional hearing could be improved with binaural apparatus. Even then the directional hearing was not as good as it was without a hearing aid. Lidén & Nordlund (1960) reported that stereophonic aids gave equally good directional hearing to that found without hearing aids. Other kinds of hearing-aid treatment gave less good results. Bender & Wiig (1960) received the impression that 12 out of the 16 children localized sound better with binaural hearing aid than without. Kodman (1961) employed a questionnaire and found that 20% of those questioned had the impression that directional hearing was improved with binaural aids as compared with monaural aids. Kuyper & de Boer (1969) found that 42 out of 55 children examined had significantly better results with two aids rather than with one It must be added that in the works quoted there is so much difference in both the methodology and the methods of evaluation employed that no direct comparisons can be made.

(c) The influence of age on the localization of noise

An investigation was made of the connection between age and the ability to localize white noise. The Spearman Rank Correlation Coefficient was found to be significantly different from 0 for groups A and B both without and with hearing aids. This indicates that for these groups of patients there is a connection between age and the ability to localize the source

of noise one must expect that the higher the age the poorer the ability to localize. Matzker & Springborn (1958) and Viehweg & Campbell (1969) came to corresponding conclusions. The latter found that directional hearing was also poorer in "very young" patients, the age of their patients ranged from 7 to 77 years. As regards groups C and D the range in age was not so great and the reports for C were noticeably worse than for the other groups over the whole range, both without and with hearing aids. Nor was the Spearman Rank Correlation Coefficient found to differ from 0 for groups C and D

COMMENTS

Our test conditions diverge to a substantial degree from listening conditions in everyday life in the ordinary way we turn towards a source of noise when we are localizing it and attempt to localize it visually This gives us the opportunity of finding the source of noise within a narrowed field even if our directional hearing is not very good. If we associate the actual noise with a visually familiar idea we can get the feeling that the source of noise can easily be localized exactly even if in isolation, the directional hearing may be rather poor This explains the fact that several of the patients who in our test conditions localized poorly were of the opinion that their directional hearing was good. In order to compare the results of our experimental measurements with the directional hearing in everyday conditions we took the patients out into the street without hearing aids. The observations made cannot be stated in figures but can briefly be described as follows: 11 of the patients turned about clearly trying to see where the noise came from when asked to localize a speaker or a vehicle the remaining 69 glanced in the right direction without any definitely noticeable hesitation. All the patients managed to achieve visual contact with the source of noise. Of the 11 patients who obviously had difficulties in localization only 5 stated that they themselves considered that localization was not quite easy Of the 69 showing no obvious difficulties, 12 remarked that it was not always easy to decide where the noise came from The patients whose directional hearing in experimental conditions had not differed from the reports of the 30 normally-hearing standard subject had none demonstrable difficulty in localizing in the street. Nor did they feel themselves that localization presented any problem.

CONCLUSION

In the 80 patients investigated we have found.

1 Impaired hearing does not necessarily entail poor directional hearing.

2. Asymmetrical hearing loss leads more frequently to poor directional hearing than approximately symmetrical hearing loss.

3 The greater the degree of one-sided hearing loss, the worse the directional hearing can be expected to be.

4 The directional hearing can be expected to be poorer with increasing age.

5 The patients own opinions about their directional hearing do not always agree with our observations.

6 The treatment with hearing aids described here cannot be expected to improve the directional hearing.

7 In cases where the hearing loss is nearly symmetrical, the directional hearing is less disturbed by binaural than by monaural hearing aid.

8 The conclusions arrived at above are valid generally for the patients as a group, but cannot be taken as absolutely valid for them individually· for this purpose they must be evaluated individually

ZUSAMMENFASSUNG

Man untersuchte bei 80 Patienten in einem echofreien Raum das Lokalisierungsvermogen fur weisses Rauschen im Horizontalplan, und zwar mit und ohne Horgerate Man kam zu dem Befund, dass herabgesetzte Horfahigkeit nicht notwendigerweise schlechtes Richtungshoren bedingte Asymmetrischer Horverlust schien eine ausgesprochenere Wirkung auf

das Richtungsgehör zu haben als annähernd symmetrischer Hörverlust. Bei am Kopf getragenen Hörapparaten des hier gebräuchlichen Typs ist eine Verbesserung des Richtungshörens nicht zu erwarten, während bei Patienten mit annähernd symmetrischem Hörverlust das Richtungsgehör durch diotische Geräte *weniger als durch monotische gestört wurde. Es ist* anzunehmen, dass das Richtungsgehör mit zunehmendem Alter abnimmt. Der Eindruck, den die Patienten selbst von ihrem Richtungsgehör hatten, stimmte nicht immer mit unseren Resultaten überein. Die beschriebenen Verhältnisse gelten nur für unsere Patienten als Gruppe gesehen und gestatten keine Schlüsse über das Richtungsgehör eines einzelnen Patienten, welches individuell beurteilt werden muss.

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Acta Otolaryng 76 121–127 1973

BIOCHEMICAL STUDIES OF OTOSCLEROSIS

Phosphohydrolase Activity in Stapedial Footplates

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(Received November 28, 1972)

Abstract. Pyrophosphate, adenosine triphosphate, and para-nitrophenylphosphate were used as substrates to determine phosphohydrolase activity on intact stapedial footplates from patients with and without otosclerosis. All phosphohydrolase activities were increased in otosclerosis, but the extent of the increase was different for each substrate. While the variation in the increase in activities in otosclerosis suggests three separate enzymes, the common properties of resistance to solubilization and similar stabilities as well as competitive inhibition between the substrates indicate a single catalytic protein with multiple phosphohydrolase activities. It appears that a qualitative change in the alkaline phosphatase may be associated with otosclerosis. The altered enzyme could be the primary factor in the etiology of otosclerosis. More likely, the organic or inorganic pyrophosphatase activities of the increased enzyme may be significant in the accretion of otosclerotic bone during progression of the disease.

Both morphologic and biochemical differences have been observed in ossicles obtained from patients with otosclerosis when compared to ossicles from patients free from otosclerosis. The most prominent biochemical differences are found in some of the enzymatic activities of the stapedes (Soifer et al. 1970; Holdsworth et al., 1971). The most striking of these is an eightfold increase in alkaline phosphatase (ALP) activity in otosclerotic stapedes when compared to controls. In spite of the magnitude of this increase in ALP activity, the increase is not sufficient to be reflected in the sera of individuals with otosclerosis (Soifer et al. 1965). The ALP activity in bone tissue, however, may be a sensitive index of foci activity.

While the precise role of ALP in bone is not known, several theories have been forwarded (Soifer et al., 1970). A recent theory states that ALP is necessary in vivo for thermodynamic pull in biosynthetic reactions (Moss et al. 1967). Whatever its role, the increase in ALP activity observed in otosclerotic bone is consistent with many other observations in which increased ALP activity is associated with the biosynthetic processes resulting in an accretion of bone. There has been a general consensus that ALP acts only on orthophosphate monoesters. Recently, however, inorganic pyrophosphatase activity has been identified by several methods as a property of purified liver, bone, and intestine ALP (Eaton & Moss, 1967 *a*; Moss, 1967). Alkaline phosphatase also catalyzes the release of inorganic phosphate from organic pyrophosphates such as adenosine di- and triphosphates (Eaton & Moss, 1967 *b*). If organic and inorganic pyrophosphatases are involved in the increased ALP activity already observed

Supported in Part by Grant 7R01 NS 09798-01-CMS, National Institute of Neurological Disease and Stroke, University of Southern California, Los Angeles, California.

in otosclerotic stapedes, the presence of these activities must be demonstrated

To date there has been no evidence to support the concept that increased activity of ALP in otosclerosis is the result of a qualitative change in the catalytic protein. Because such alterations of ALP have been reported in other systems (Cox et al 1971), this possibility was considered.

This report presents data confirming the presence of inorganic pyrophosphatase (PPase) and adenosine triphosphatase (ATPase) activities in stapedial footplates. These activities are also increased in otosclerosis. Evidence is presented suggesting that the PPase ATPase and para-nitrophenylphosphatase (PNPPase) activities are characteristic of a single catalytic protein. This single catalytic protein appears to be altered qualitatively as well as quantitatively in otosclerosis.

MATERIAL AND METHOD

Stapedes were obtained from patients with clinically diagnosed otosclerosis, confirmed at surgery Stapedes removed at autopsy from non-otosclerotic individuals were used as con ols. Surgical samples were rinsed quickly ,Ringer s lactate solution to remove any ontaminating blood. The specimens were then blotted well, wrapped in foil, quickfrozen and stored at −15 C.

The stapedial footplate was fractured free from the crura with a microknife and quickly weighed to the nearest 0 01 mg. Cold Tris-(hydroxymethyl)aminomethane hydroclonde (Tris-HCl) buffer 0 1 M pH 8.5 was used to bathe the tissue to prevent tissue drying and loss of enzyme activities. Alkaline phosphatase activity was previously discovered to be resistant to extraction from powdered stapedes by buffer solution apparently remaining tightly bound to bone although other enzymes were readily extracted (Soifer et al. 1969)

In experiments without exogenous substrate, we had observed that inorganic phosphate (P_i) was released into solution. This rate of endogenous release was determined as follows: prior to assaying for phosphohydrolase activity the suspension of bone was equilibrated to 37 C in Tris-HCl buffer the buffer was replaced with 3 ml of fresh buffer (Tris-HCl buffer 0 1 M pH 8.5) also equilibrated to 37 C, and the rate of endogenous P_i re lease obtained by removing aliquots of buffer at 0, 30 and 60 min and determining soluble inorganic phosphate

Following this procedure, 3 ml of solution containing 3 3 mM pyrophosphate (P-P), 1.0 mM Mg^{++} and 100 mM Tris-HCl, pH 8.5 was added to initiate the pyrophosphatase reaction. Aliquots were removed at various time intervals and assayed for P_i released. At the termination of the assay the remaining solution was removed from the bone chips and discarded. The bone was washed with new warm buffer without substrate and after the bone had been allowed to settle the supernatant fluid was removed and discarded. The bone chips were then used for the next procedure employing another substrate.

The determination of ATPase activity was made using 2.0 mM Tris-ATP pH 8.5 20.0 mM Mg^{2+} and 70 mM Tris buffer pH 8.5 (Nagode, in preparation).

Following the determination of ATPase activity the bone tissue was washed again in Tris-HCl buffer and tissue assayed for ALP activity using 15 0 mM *p*-nitrophenyl phosphate, 0.5 mM Mg^{2+} and 1 0 M diethanolamine-HCl buffer at pH 9 8 (Hausamen et al. 1967). However instead of determining the P_i released as in the PPase and ATPase determinations, the hydrolysis of PNPP was determined colorimetrically at 400 nanometers after removing the yellow solution from the bone tissue.

All of the above reactions were carried out with continuous vigorous mixing in multihead vortex mixers for a period of 1 hour for endogenous (no substrate added) phosphate release and ALP activity and for a period of

Table I. *Phosphohydrolase activity*[a]

Substrates. P-P pyrophosphate; ATP adenosine triphosphate; PNPP para-nitrophenyl phosphate. Each value represents the activity as determined on a single footplate. Each experiment consists of 3 assays with different substrates and accomplished with the same, single footplate

	Substrate					
	P-P		ATP		PNPP	
Experiment	Oto[b]	Control	Oto	Control	Oto	Control
1	18	10	—	18	112	53
2	25	5	—	18	—	79
3	15	5	—	20	176	45
4	14	13	—	25	124	40
5	12	17	33	30	118	64
6	19	13	36	12	180	40
7	11	11	27	13	415	48
8	20	7	25	1	307	41
9	18	12	37	9	127	78
10	—	11	—	18	—	63
Average ± S.D.	16 ± 4 4	10 ± 3.8	32 ± 5.4	18 ± 6.4	195 ± 110	55 ± 15
Student *t*-test	$t = 3\ 4$[d]		$t = 4.5$[d]		$t = 3\ 6$[d]	

Activities are expressed 10^{-6} micromoles of substrate converted per min per mg.
[b] Otosclerotic.
Indicates assays using a given substrate were not performed.
Significant at $p < 0.005$.

3 hours for inorganic pyrophosphatase and ATPase assays. All reactions were carried out in a temperature controlled room at 37 C. Neither washing nor shaking with buffer or substrate solutions solubilized any of the phosphohydrolase activities. *Phosphorus release* without substrate and all enzyme activities are expressed as micromoles of substrate converted per min per mg wet weight of bone. Note that for pyrophosphate 2 moles of phosphate are released per mole of pyrophosphate hydrolyzed.

RESULT

Release of P_i in the absence of exogenous substrate was 0.05 (0 02–0.1) nanomoles per min per mg tissue for non-otosclerotic foot plates and 0.07 (0.02–0.12) nanomoles per min per mg in otosclerotic footplates. This represents less than 10% of the P0 released with either pyrophosphate or ATP. The P P and ATP phosphohydrolase activities presented in Table I have not been corrected for endogenous activity since all or part of the P_i release without exogenous substrate may be enzymatic in origin, in which case the endogenous P_i release should be considered as part of the total enzyme activity. Heating the tissue at 100 C for 30 min completely eliminated phosphohydrolase activity as determined using PNPP as the substrate and decreased the endogenous release of P_i by approximately 60%. The remaining 40% may be due to non-enzymatic hydrolysis and/or release of P_i. However one can only speculate because the heating itself could be responsible for the remaining 40% released. In any case, the rate of P_i release without exogenous substrate is so similar in otosclerotic and non-otosclerotic foot plates that the lack of a correction for endogenous P_i release does not alter or invalidate the difference in phosphohydrolase activity observed (Table I). The endogenous release of P_i does not have to be considered when PNPP is used as substrate because the nitrophenol is determined instead of P_i. Linearity was obtained in reactions with each of the three substrates, P P ATP and PNPP for at least 3 hours.

Table II. *Comparison of two methods for the determination of phosphohydrolase activity*

Activities are expressed × 10⁻⁴ micromoles of para-nitrophenylphosphate converted per min per mg. The activity was determined first using buffer containing glycine, then using another containing diethanolamine

	Phosphohydrolase Activity (PNPP)			
	Non-otosclerotic		Otosclerotic	
Pool[a]	Glycine	Diethanol-amine	Glycine	Diethanol-amine
1	6	38	140	133
2	8	55	90	148
3	7	38	64	260
4	6	67	54	150

[a] The pool represents a minimum of 10 stapedes.

When bone chips were stored in Tris-HCl buffer at 4 C the phosphohydrolase activity towards P P ATP and PNPP remained con stant for 3 days or more. The identical stability of the catalytic activity for the three substrates is suggestive of a single enzyme with broad specificity and reflects the dif ficulty in solubilizing the enzyme(s) from footplates. However the ratio of catalytic ac tivity towards P P ATP and PNPP in control tissue is 1 2 5 respectively but is ~2 12 in otosclerotic tissue (Table I). Fur the increase in catalytic activity towards *P P ATP and PNPP in otosclerotic tissues* over controls is 60% 78% and 255% These results suggest either a quantitative change in two or more separate enzymes, or a qualitative change in a single enzyme reflecting a greater change of activity towards PNPP

The magnitude of the differences in ALP activities between otosclerotic and non-otosclerotic stapedes was measured using two methods for determining PNPP phosphohydrolase activity The same tissues were used in each assay The results in Table II show the same large differences in ALP activity between otosclerotic and control tissues with PNPP and glycine buffer as previously re ported (Soifer et al 1969 and 1970) Using the PNPP-diethanolamine assay (described in Method") the average difference is approximately the same between otosclerotic and control footplates (compare results Table I and PNPP-diethanolamine Table II). Substantially less activity was observed (6–11 fold less) in non-otosclerotic stapedes with glycine buffer than with the diethanolamine buffer (Table II) The difference between systems for the otosclerotic stapedes is much less re markable 3–4 fold less activity was observed with glycine buffer The differing effects of the two buffer systems on phosphohydrolase activity in the otosclerotic and non-otosclerotic tissues may be explained by the presence of an altered phosphohydrolase enzyme in the otosclerotic tissue.

Phosphohydrolase activities are elevated in otosclerosis using P P and ATP as substrates, but the increase was greatest for PNPP suggesting two or more distinct enzymes (Table I). Activity was subsequently determined with two substrates present in the incubation mixture. The reactions were car ried out at pH 8 5 in Tris-HCl buffer This buffer system was used to insure solubility of the pyrophosphate substrate which, in the presence of magnesium precipitates at higher pH values. If separate enzymes in bone are involved in the hydrolysis of these phosphate esters, additive activities should be observed. *If only a single enzyme with broad specificity* is involved the presence of another substrate would act as a competitive inhibitor of the

Table III *Effect of two substrates on phosphohydrolase activity*

Activities are expressed 10⁻⁴ delta optical density per mg per reaction

Stapedes	*PNPP*	*PNPP + P – P*	*PNPP + P – P*
Non-otosclerotic			
Pool 1	3.0	1 7	0.57
Pool 2	6 0	3 6	0 60
Otosclerotic			
Pool 1	46.0	28.0	0.61
Pool 2	13.0	8 3	0 64

initial substrate. Results in Table III show the effect of pyrophosphate on phosphohydrolase activity towards PNPP Phosphohydrolase activity on PNPP was decreased by 40% in the presence of pyrophosphate suggesting a single common enzyme. The per cent decrease was essentially the same for normal and otosclerotic stapedes.

Methylene diphosphonate, the carbon analogue of pyrophosphate, is not hydrolyzed by phosphohydrolase and is an inhibitor of the enzyme. Its effect on the phosphohydrolase activity of otosclerotic and non-otosclerotic bone was determined to see if it might have a selective effect on activity Inhibition was observed to the same degree in both otosclerotic and non-otosclerotic stapedes (Table IV). Methylene diphosphonate was also used in concentrations simulating P P in order to determine the effect on the P_i release without exogenous substrate The effect was very similar to the inhibition observed in the presence of hydrolyzable substrate.

Prior exposure of the bone tissue to glycine buffer prevented further inhibition of phosphohydrolysis activity by MDP with otosclerotic tissue, but normal inhibition by MDP was observed with non-otosclerotic tissue (Table V) The phosphohydrolase activity was determined initially in the glycine buffer which was then removed by thorough washing with Tris-HCl buffer The assay was then repeated in the diethanolamine buffer in the presence and absence of MDP The activities for the controls are comparable to those observed in diethanolamine buffer without prior glycine treatment The only observable difference is the absence of sensitivity of the otosclerotic tissue to MDP inhibition. Glycine inhibition of bone phosphohydrolase has been described (Bodansky 1946)

Table IV *The effect of methylene diphosphonate on phosphohydrolase activity (PNPP)*

Activities are expressed 10^{-4} delta optical density per min per mg. Control represents activity with para-nitrophenylphosphate without methylene diphosphonate (MDP)

	Non-otosclerotic		Otosclerotic	
Pool*	Control	MDP	Control	MDP
1	20	8	380	106
2	26	7	226	75

The pool represents a minimum of 10 stapedes.

Table V *The effect of MDP on phosphohydrolase activity after exposure to glycine*

Activities are expressed 10^{-4} micromoles of para-nitrophenylphosphate converted per min per mg (Control) and in the presence of methylene diphosphonate (MDP)

	Non-otosclerotic		Otosclerotic	
Pool*	Control	+ MDP	Control	+ MDP
1	38	18	133	125
2	55	22	148	144

The pool represents a minimum of 10 stapedes. The phosphohydrolase activity was measured using a diethanolamine buffer solution after a previous exposure of the bone tissue to glycine buffer

COMMENT

Much of the Mg^{+2} ATPase and all of the Ca^{+2} ATPase and ALP activity have been shown by parallel inhibition studies to represent different measures of the activity of a single enzyme using purified ALP enzyme preparations from chick intestine (Haussler et al., 1970) In this study isolation of the molecular species containing ALP was not feasible because of the small amount of tissue and the insolubility of ALP Reagent mixtures such as butanol-water Triton X 100 etc. which have been traditionally used to solubilize or extract ALP from tissues, including bone fail to remove more than one tenth of one per cent of the activity from either powders of ossicles or intact stapedial foot plates (unpublished observations). However the true efficiency of solubilization of bone ALP activity from sources other than ossicles has not been reported. Generally when extracting an enzyme for purification there appears to be little effort in efficient isolation when large amounts of activity are ob-

tained, even though that activity may represent only a small fraction of the enzyme associated with the tissue itself The resistance of ALP to extraction may not be unique to ossicular bone tissue A similar resistance of extraction ALP was observed with chips of cortical bone (Soifer et al. 1969) The continuous presence of buffer solutions while readily extracting soluble enzymes, failed to extract the ALP activity from bone tissue either cortical bone or stapedial footplates. With the insolubility stability and level of ALP activity of the bound enzyme in mind, the assays were carried out with and without inhibitors in buffer solutions containing bone itself The use of single footplates for assay of the tightly bound ALP has the advantage of conserving tissue, as intact otosclerotic stapedial footplates are rarely obtained.

As results show phosphohydrolase activity was observed with pyrophosphate, adenosine triphosphate and para-nitrophenylphosphate in both otosclerotic and control stapedial foot plates. The data obtained with individual foot plates are more variable however than previous data obtained with powdered pools of stapedes on PNPP Pooling the tissue obviously averages out the variations of the individual footplates. It has been suggested (Soifer et al. 1970) that since there appears to be a lack of complete correlation between ALP activity and bone growth as estimated by the degree of otosclerotic involvement, the ALP activity varies with the number and activity of otosclerotic foci at the time of sampling. Accordingly an occasional high alkaline phosphatase activity observed in a "normal tissue may be due to a number of very active foci with no obvious gross involvement, and a low value in an otosclerotic foot plate may be the result of quiescent foci.

Despite the variations, inorganic PPase and ATPase activities are significantly elevated in otosclerotic stapedial footplates (Table I). The ALP activity in otosclerotic stapedes is 450% greater than controls, while the corresponding increase in inorganic PPase and ATPase activity is considerably less 60% and 78% respectively

All phosphohydrolase activities on PNPP presented in Table I are much higher than those reported previously either on pooled powders or on individual footplates (Soifer et al. 1969 and 1970) An assay procedure with diethanolamine buffer was used for the determinations reported here while previous determinations were made in the presence of glycine buffer It has been reported that glycine at low concentrations inhibits bone alkaline phosphatase (Bodansky 1946).

There is an indication from the data in Table II that glycine may have less inhibitory effect on ALP in otosclerotic stapedes than in the ALP from normal stapedes. This would suggest that the ALP enzyme in the otosclerotic bone may be altered in some way that it is no longer subject to the same degree of control by glycine as the ALP enzyme from normal bone.

Methylene diphosphonate a competitive inhibitor of ALP inhibits ALP from normal and otosclerotic bone to the same degree (Table IV) However after exposure of the tissue to glycine, the inhibitory effect of MDP is no longer observed on the ALP in otosclerotic bone. While no mechanism can be offered for this effect, it would also suggest that the ALP enzyme in the otosclerotic bone was altered in some way from the normal ALP enzyme.

The ratio of PPase activity to ALP activity in otosclerotic bone is about 1 16 whereas the ratio in normal bone is about 1 5 If one assumes that both activities are contained within a single protein as suggested above, the enzyme must necessarily be altered in some way to account for the change in the ratio of these two activities.

The phosphohydrolase activities measured on the three different substrates have certain properties which would indicate that the three catalytic activities are characteristic of one protein All three are difficult to solubilize, all are stable to the same degree over a pe-

riod of days, and all are increased in otosclerotic bone. Further the marked inhibition of the activity on PNPP when it was determined in the presence of inorganic pyrophosphate (Table III) would suggest that the two substrates were *competing for the same* site on a single enzyme. Distinct molecular species would show P_i released equal to the sum of that which would be released from each enzyme alone.

Although it is possible that separate PPase, ATPase, and PNPPase enzymes are involved, the likelihood of a single species with multiple specificity seems more reasonable. Whichever explanation proves correct it is clear *that the three phosphohydrolase ac*tivities are present and significantly increased in otosclerotic stapedes. This could be physiologically significant since the increase in activities appears to be compatible with the accretion of bone in otosclerosis.

ACKNOWLEDGMENT

We express our sincere appreciation to Carole Steinmeyer for her assistance with the assay preparations. We thank the Department of Pathology Good Samaritan Hospital for their co-operative efforts in *obtaining control stapedes.*

ZUSAMMENFASSUNG

Als Substrate zur Aktivitätsbestimmung der Phosphohydrolasen intakter Gehörsteigbügelplatten von Patienten mit oder ohne Otosklerose wurden Pyrophosphat, Adenosintriphosphat und p-Nitrophenylphosphat angewandt. *Alle Phosphohydrolase-Aktivitäten* waren *bei Otosklerose erhöht, die Grösse der Zunahme* hing jedoch vom verwandten Substrat ab. Die Verschiedenheit der Aktivitätszunahme bei Otosklerose liess auf drei separate Enzyme schliessen, existierten nicht einige gemeinsame Fermenteigenschaften, nämlich Unlöslichkeit vom Knochen, Ähnlichkeit in der Stabilität und kompetitive Hemmung zwischen den Substraten, die ein einziges katalytisches Enzym mit multiplen Phosphohydrolase-Aktivitäten annehmen lassen. Vermutlich ist eine qualitative Veränderung in der alkalischen Phosphatase mit Otosklerose assoziiert. Vielleicht ist das chemisch veränderte Enzym der eigentliche Grund für die Entwicklung der Otosklerose. Noch wahrscheinlicher ist, dass eine quantitative Zunahme der organischen oder inorganischen Pyrophosphatasen aufgrund anderer Ursachen zu einer beträchtlichen Vermehrung an sklerotischen Knochen während der Krankheitsphase führt.

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Acta Otolaryng 76. 128–135 1973

NEOMYCIN OTOTOXICITY AND THE COCHLEAR EFFERENTS

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(Received October 13 1972)

Abstract The effects of neomycin on cochlear histology were correlated with its effect on contralateral olivo-cochlear bundle (COCB) stimulation in cats given one subcutaneous injection of neomycin per day (100 mg/kg/day) for 0 (controls), 4 6, 7 10 and 12 days. Enhancement of COCB stimulation effects occurs first, the highest incidence being found after 6 and 7 days of neomycin treatment. This is then followed by a block of COCB stimulation effects which is greatest at 10 and 12 days of neomycin treatment. *There is minimal damage to the cochleas of these* animals, indicating that enhancement and block of COCB stimulation effects may be due to a pharmacological action on the efferent synapse which occurs *before* extensive ototoxicity develops. This pharmacological synaptic activity of neomycin could be playing a role in the genesis of ototoxicity

honen (1965) found a correlation between the degree of density of efferent innervation of the guinea pig cochlea and the pattern of *hair cell degeneration produced by neomycin* and kanamycin. Because of this finding he suggested that the ototoxic effect may be due to interference with efferent function.

Intra-arterial intraperitoneal, or iontophoretic administration of neomycin kanamycin, streptomycin and dihydrostreptomycin *produces decreases in* N_1 *and/or increases in* microphonics when injected into cats or guinea pigs (Blair Simmons et al. 1960 Sohmer & Feinmesser 1965 Brown 1968). These findings indicate that the ototoxic antibiotics can exert a weak agonistic action at the efferent synapse when injected acutely

Brown (1968), using cats, found that acute *intra-arterial administration of neomycin,* kanamycin, and streptomycin produced no changes in the N_1 suppression and microphonic augmentation obtained by stimulating the contralateral cochlear efferents (contralateral olivo-cochlear bundle, COCB) However he (Brown, 1972) did find that chronic administration of neomycin and kanamycin *to cats* for 8 days produced a block in some of the neomycin animals and an increased effectiveness of COCB stimulation in some of the neomycin animals as well as in the kanamycin animals. (Histologic studies were not performed on any of these animals.)

The purpose of this study was to correlate the effects of neomycin on cochlear histology with its effect on COCB stimulation. Neomycin was given to cats for 4 to 12 days, followed by testing the effects of COCB stimulation In conjunction, histologic evaluation of the cochleas was performed.

METHOD

Thirty-two healthy adult cats, 9 males and 23 females, weighing between 1 8 and 4.5 kg were used. The animals were anesthetized with an intraperitoneal injection of Dial-Urethane (0.65 ml/kg).

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Animals were prepared for round window recording from the left cochlea and stimulation of the COCB in a manner similar to that described by Daigneault & Brown (1969).

N_1 suppression was used to gauge the efficacy of COCB stimulation. Input–output functions, as described by Brown (1972) were obtained and used to accumulate data on N_1 suppression in terms of equivalent decibels attenuation of the sound energy (reference 1.0 V = 0.0 dB).

Efficacy of COCB stimulation was determined by ascertaining the amount of N_1 suppression produced at 400 Hz of stimulation, using 8, 15, 30 and 45 V of stimulation.

Statistical analyses were performed using either unpaired Student "*t*" tests (as described by Dixon & Massey 1957) or tests of significance on enumeration data when cell frequencies are small (Fisher's exact test as described by Batson, 1956).

At the termination of most experiments, the round and oval windows of both cochleas were opened, the cochleas slowly perfused with 0.50 cc of a zinc iodide/osmium tetroxide solution (Engström et al., 1966) and the cochleas removed and placed in a solution of zinc iodide/osmium tetroxide for 12–18 hours. The cochleas were then washed gently with distilled water and placed in buffered formaldehyde until histologic studies could be performed on them.

When histological evaluation of the cochleas was to be performed, the petrosal bone was isolated then decalcified with 20% formic acid. The bone was labeled in a paper tea bag and placed in the decalcifying solution under constant stirring with a magnetic stirrer until the bone was softened (3 to 6 days). The decalcified bone was then dissected to prepare surface preparations using fine forceps with the aid of a dissecting microscope. The decalcified bone was carefully removed to expose the membranous cochlea *in toto*. Then the tectorium was removed and two representative sections of the basal, middle and apical turns were removed using iridectomy scissors. These sections were mounted on a slide in a water-soluble medium (Paragon Mounting Medium. Paragon C. & C. Co., Inc., N.Y., N.Y.) and covered with a cover slip. Care was taken to be certain that the tissue was placed upright for proper visualization of the hair cells. The cells were then studied with the aid of a microscope and judged on a four-to-zero scale based upon the presence, absence, or distortion of hairs, incidence of outer hair cell destruction and the condition of the inner hair cells.

The remaining portion of the cochlea (the modiolus) was then dehydrated in alcohol and run through the Technitron embedding procedure. The paraffin blocks were serially sectioned at 15 μm from the apex to the basal turn and counterstained with hematoxylin and eosin. These slides were studied to identify the condition of the spiral ganglion afferents and efferents (intraganglionic spiral bundles) at the appropriate levels of the turns of the cochlea. The neurons were also judged on a four-to-zero scale based upon the incidence of missing neurons and the degree of pyknosis of the neuron and its nucleus. An estimate of the density of the afferent and efferent fibers coursing from or through the spiral ganglia was also noted in each slide studied.

Only one person made all the histological observations to reduce the variation in judgment. The animal tissues were coded so that the histologic evaluation was done without any knowledge as to the history of the specimen. (The measurements of physiological and histological parts of these experiments were performed in separate laboratories and the person making the histological evaluations was kept blind to the type of treatment rendered to the animals.)

The slides were evaluated using the following scoring system: 4, excellent condition; 3, good; 2, fair; 1, poor; 0, artifact. The value for each slide was then totaled and the cochlea given a grade representing the degree of intoxication of hair cells and spiral ganglion cells, afferent and efferent fibers. When the

Table I *Equivalent decibels attenuation of N_1 produced by COCB stimulation*

Days of neomycin treatment	Mean dB attenuation ± S.E.M. (no. of animals)			
	Weakest mA (8V)	Weak mA (15V)	Strong mA (30V)	Strongest mA (45V)
Controls	6 03±2.48 (6)	12.2±3.68 (6)	18 1±2.34 (6)	18.4±1 14 (6)
4	13.9±5.93 (3)	14.6±6.67 (3)	20 2±2.77 (3)	21.9±1 43 (3)
6 Enhanced	13.0±5 40 (2)	16.5±4.10 (2)	26.4±0.75 (2) $p = 0.05$[a]	29.5±0.85 (2) $p < 0.005$[a]
6 Not enhanced	6.43±3 73 (3)	15.9±0 75 (3)	17 8±0.87 (3)	17.5±0.62 (3)
7	6.00±5 60 (2)	10.1±1.50 (2)	18.1±1 79 (2)	20 0±0 90 (2)
Brown (1972)[b]				
Controls	8.31±1 73 (9)	16.8±0.52[c] (9)	18.6±1.06 (9)	19.[illegible]±1.60 (9)
8 Blocked	2.22±0.90 (6) $p < 0.01$	5 40±2.37 (4) $p < 0.005$[a]	6.80±2.62 (4) $p < 0.005$[a]	7 17±1 76 (6) $p < 0.005$[a]
8 No block	16.3±2.38 (7) $p < 0.01$	21 1±1 18 (7) $p < 0.005$[a]	20 7±2.15 (7)	21 4±2.36 (7)
10 Blocked	1.53±0.82 (3)	3 63±[illegible].00 (3)	6.97±4.01 (3) $p < 0.0$[illegible]5[a]	10.5±2.67 (3) $p < 0 01$
10 No block	9 65±2.85 (2)	15.3±1.90 (2)	16.7±3.50 (2)	18.0±3.95 (2)
1[illegible]	0 (1)	0 (1)	0 (1)	0 (1)

All p values obtained by comparing the appropriate control dB attenuations with others of the same column. Only p values equal to or less than 0.05 are listed.

[b] The following controls and 8-day animals are reprinted, with permission, from Brown (1972) for purposes of comparison.

Decimal incorrectly placed in original publication.

entire study was finished, the scores were placed on a master sheet and the score sheet deciphered

RESULT

[illegible] five cats received one subcutaneous [illegible] of neomycin sulfate (Sigma Chemical Co (pfs) St. Louis, Mo lot no. 059 B 2130) per day (100 mg/kg/day) for varying periods of time 4 received neomycin for 4 days, 6 for 6 days, 2 for 7 days, 9 for 10 days and 4 for 12 days. The day after the specified treatment ended, efficacy of COCB stimulation was ascertained. Seven untreated animals served as controls. The dates of experiments on the various series of animals were interspersed over an 8 month period.

A variety of circumstances prevented evaluation of COCB stimulation effects on some of the animals in the study- (1) one of the controls suffered a basilar skull fracture while the stereotactic ear bars were being placed, (2) blood pooled around the stereotactic electrode placed in one of the 4-day animals, preventing evaluation of COCB stimulation, (3) 2 animals died one of the 10-day cats (secondary to treatment for a tapeworm discovered after the experiment began) and one of the 12-day cats (onset of gastroenteritis after neomycin treatment began), and (4) 6 other neomycin animals had no recordable N_1 on the day of COCB stimulation. Therefore evaluation of the efficacy of COCB stimulation was carried out on 6 of the control animals, 3 of the 4-day animals, 5 of the 6-day animals, both the 7-day animals, 5 of the 10-day animals and 1 of the 12-day animals.

The equivalent decibels attenuation of N_1 obtained at 400 Hz with the 4 currents of stimulation are presented in Table I The currents obtained at the 4 voltages of stimulation were not significantly different from those obtained in an earlier series of experiments (Brown, 1972) and therefore are not presented in tabular form.

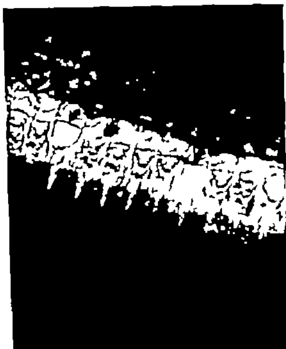


Fig. 1 Surface preparation of a basal turn segment from an animal mildly intoxicated with neomycin (6 days pretreatment). (670) Some of the outer hair cells in the first row are missing (←) and some of the hairs on the remaining outer hair cells are distorted. The inner hair cells seen in a row above the missing outer hair cells are not visibly affected.

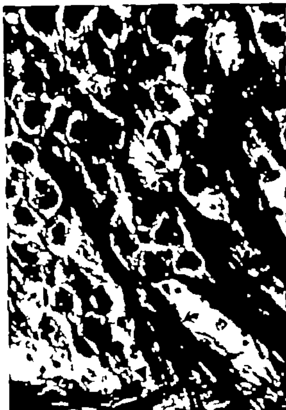


Fig. 2 Spiral ganglion of the basal turn from a control animal. (335) Note the density of the spiral ganglion cells, afferents (←) and efferents (◄).

In 2 of the recordable 6-day neomycin-treated animals, the most effective COCB stimulation was more effective than those of any of the control animals (the 6 days, enhanced category). These animals were placed in a separate category because use of Fisher's exact test yielded a two-tailed probability of much less than 0.05 ($p = 0.00607$) indicating that the enhancement seen did not occur by chance or result from better placement of the stereotactic electrode. In 3 of the recordable 10-day animals, the effectiveness of COCB stimulation was less than in the controls (the 10-day blocked category). They were placed in a separate category because the two-tailed probability was less than 0.05 ($p = 0.01819$). The one usable 12-day animal also exhibited a complete block of COCB stimulation effects.

Representative photomicrographs of hair cell surface preparations and spiral ganglion sections of control and neomycin-treated animals are presented in Figs. 1–3. The cell damage produced was most marked in the outer hair cell. The first indication of damage that was recognized was the distortion of hairs on the outer hair cell. A greater degree of intoxication was evidenced by hairs being lost in the outer hair cell population. The highest degree of intoxication was indicated by the loss of increasing amounts of the outer hair cells. In all specimens which exhibited signs of intoxication, the damage to the outer hair cells was more severe at the basal turn of the cochlea, decreasing in severity upward to the apex. In the most severely intoxicated animals, approximately 20% of the outer hair cells in the basal turn were missing. Damage

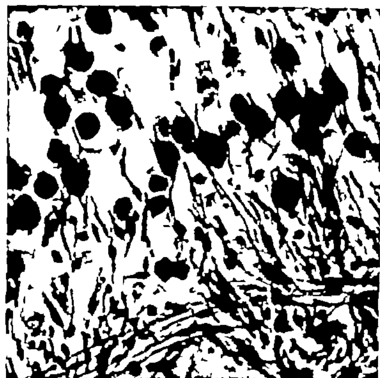


Fig 3 Spiral ganglion of the basal turn from an animal severely intoxicated with neomycin (10 days pretreatment, no recordable potential). (×335) Note the greatly reduced neuron and afferent fiber (←) population. Some efferents (◄) are still present.

to the inner hair cell was minimal even in the most severely intoxicated animals (when seen, it was evidenced by distortion and loss of hairs).

The earliest indication of damage to the spiral ganglion was the presence of pyknotic neurons with different staining properties. As intoxication increased, an increasing loss neurons occurred with an associated decrease of afferent fiber concentration. The efferents (intraganglionic spiral bundles) were seldom seen to be altered to the extent of the afferent fiber changes. There were some instances of a depressed concentration of efferent fibers but it was not a consistent finding even in the severely intoxicated animals. As with the outer hair cells, the damage to the neurons and afferent fibers was greater in the basal turn of the cochlea.

Scores obtained from histologic examination of the left cochleas, the dB of the acoustic stimulus used when stimulating the COCB and the N_1 height at this acoustic stimulus and at 0 dB are presented in Table II. These data indicate that cochlear damage occurs due to treatment with neomycin.

Animals in which cochlear potentials could not be recorded had generally lower histology scores than the recordable animals did (the 6- and 10-day animals) although there were exceptions (the 12-day animals). At first appearance, it would seem that all of the no-recordable potential animals should have lower histology scores than the usable animals. However undetected middle ear disease processes (otosclerosis, for example) and excessive attenuation of the acoustic stimulus due to ear bar placement coupled with a certain degree of cochlear damage produced by neomycin could be responsible for the animals with the higher histology scores not recording.

Histology scores on the hair cells of the neomycin animals are less than and significantly different from the controls with one exception those of the 10-day not-blocked category (Table II) However the histology scores on the neurons, afferents, and efferents of the neomycin animals are not significantly different from the controls, except for the 10-day no-recordable potential category (Table II). In addition, there is no con-

Table II. *Effects of neomycin treatment on the histology scores of left cochleas, dB of the acoustic stimulus used when stimulating the COCB N_1 height at this acoustic stimulus and at 0 dB*

		Histology scores ± S.E.M.				
Days of neomycin treatment	No. of animals	Hair cells	Neurons, afferents & efferents	Mean dB acoustic stimulation ± S.E.M.	N_1 Height (μV) at this acous. stim. ± S.E.M.	N_1 Height (μV) at 0 dB[a] ± S.E.M.
Controls	6	4.0 ± 0.0	3.4 ± 0.3	+2.0 ± 6.6	333 ± 26	272 ± 64
4	3	Not done	Not done	+24.7 ± 4.7 $p < 0.05^{b}$	346 ± 31	90 ± 31 $p < 0.05^{b}$
6, Enhanced	2	3.5 ± 0.0 $p < 0.005^{b}$	3.8 ± 0.3	+4.0 ± 10.0	438 ± 37 $p < 0.05^{b}$	458 ± 153
6, Not enhanced	3	3.0 ± 0.3 $p < 0.005^{b}$	4.0 ± 0.0	+2.7 ± 16.2	314 ± 35	278 ± 151
6, No recordable potential	1	3.0 $p < 0.005^{b}$	3.0	—	—	—
7	2	3.3 ± 0.3 $p < 0.005^{b}$	3.5 ± 0.5	+27.0 ± 7.0 $p < 0.005^{b}$	173 ± 55 $p < 0.025^{b}$	28 ± 28 $p < 0.05^{b}$
Brown (1972)[c]						
Controls	9	Not done	Not done	+8.44 ± 3.77	273 ± 49	134 ± 43
8, Blocked	6	Not done	Not done	−13.7 ± 7.37	319 ± 54	173 ± 48
8, No block	7	Not done	Not done	+12.9 ± 3.32	295 ± 51	170 ± 77
10, Blocked	3	2.7 ± 0.9 $p < 0.05$	3.0 ± 0.3	+25.7 ± 6.6 $p < 0.01^{b}$	228 ± 64	58 ± 58 $p < 0.05^{b}$
10, Not blocked	2	3.8 ± 0.3	3.8 ± 0.3	+28.5 ± 8.5 $p < 0.01^{b}$	338 ± 114	136 ± 136
10, No recordable potential	3	2.2 ± 0.4 $p < 0.005^{b}$	2.3 ± 0.3 $p < 0.05^{b}$	—	—	—
12	1	1.5 $p < 0.005^{b}$	[illegible]5	+34.0 $p < 0.01^{b}$	129 $p < 0.025^{b}$	0 $p < 0.025^{b}$
12, No recordable potential	2	3.0 ± 0.5 $p < 0.005^{b}$	3.5 ± 0.0	—	—	—

The only dB common to the input–output of all the animals at the various days of neomycin treatment.
p values obtained by comparing the control values with others of the same column. Only p values less than 0.05 are listed.
The following controls and 8-day animals are reprinted, with permission, from Brown (1972) for the purposes of comparison.

sistent decrease in either hair cell or neural element scores as the treatment with neomycin is prolonged. Thus, it seems that the neomycin treatment used in these experiments produces minimal ototoxicity.

Some cochlear physiological parameters of the recordable animals were tabulated in order to determine if they correlated with the histologic damage present (Table II). There is a general tendency for the intensity of acoustic stimuli used when stimulating the COCB to be higher in the neomycin animals than that of the controls. However there is no progressive increase as the treatment with neomycin is prolonged. In addition, there are no consistent changes of N_1 amplitude produced by neomycin treatment. Therefore the physiological parameters do correlate grossly with the histology scores and indicate that minimal damage to the cochlea is produced by neomycin in the recordable animals.

DISCUSSION

Chronic treatment with neomycin, at doses producing definite histotoxicity resulted in modification of the effects of COCB stimulation. When the cochlear histology and the

Table III *Summary of histology scores and responses to COCB stimulation at different intervals of neomycin treatment*

Days of neomycin treatment	No. of animals	Histology scores ± S.E.M. Hair cells	Histology scores ± S.E.M. Neurons, afferents & efferents	COCB stimulation effects (% of total) Normal	COCB stimulation effects (% of total) Enhancement	COCB stimulation effects (% of total) Block	COCB stimulation effects (% of total) No recordable potential
0	6	4.0±0.0	3.4±0.3	100	0	0	0
4	3	Not done	Not done	100	0	0	0
6 & 7	8	3.2±0.1 $p<0.005$[b]	3.7±0.2	63	25	0	12
8[a]	20	Not done	Not done	30	5[c]	30	35
10 & 12	11	2.7±0.3 $p<0.005$[b]	3.0±0.2	18	0	36	46

[a] Data reproduced, with permission, from Brown (1972) for the purpose of comparison.
[b] *p* values obtained by comparing the control values with others of the same column.
p values greater than 0.05 are not listed.
[c] Only one of the 8-day animals exhibited enhancement at all currents of COCB stimulation even though all of the 8-day no-block animals exhibited it at the lower currents of stimulation when taken as a group.

responses of neomycin-treated animals to COCB stimulation are summarized (Table III) a definite pattern does emerge. Enhancement of COCB stimulation effects occurs first, reaching a peak at 6 and 7 days of neomycin treatment. This is then followed by a block of COCB stimulation which is greatest at 10 and 12 days.

Even though cochlear damage is sustained during neomycin treatment, there does not [appe]ar to be any correlation between the [am]ount of damage sustained and the development of enhancement or block of COCB stimulation effects. Hair cell damage is found in the 6 & 7 and 10 & 12 day neomycin-treated animals but there is no significant difference between them (Table III) The neuron afferent and efferent scores after neomycin treatment are not significantly different from those of the controls (Table III) In fact the overall histology scores of all the neomycin-treated animals are rated 2.7 or better 3.0 being defined as good. In addition no significant difference is found in the histology scores when comparing the 6 day enhanced category to that of the 10 day blocked category (Table II) This may indicate that enhancement and block of COCB stimulation effects are due to a pharmacological action of neomycin on the efferent synapse which occurs *before* extensive ototoxicity develops.

The development of enhancement followed by a block as exposure to neomycin continues could be due to the drug interfering with release of the mediator at the presynaptic terminals of the cochlear efferents. Using this rationale of a pharmocologic block, the enhancement of COCB stimulation effects would be due to the block being strong enough to prevent normal release of the mediator thereby causing a degree of denervation hypersensitivity but weak enough to be over ridden by optimal electrical stimulation of the efferents. (Denervation hypersensitivity to acetylcholine a candidate for mediator at the cochlear efferent terminals, does develop when the cochlear efferents are sectioned Daigneault & Brown, 1969· Daigneault & Blanton, 1971)

The COCB blocked category would then be due to this block becoming stronger as exposure to neomycin continues since the ototoxic *Streptomyces* antibiotics do accumulate in cochlear fluids (Voldrich 1965 Wersäll & Lundquist, 1968) Animals at the different intervals which respond in a "normal" way to COCB stimulation would be in a transi-

tion state between the enhanced and blocked categories.

One cannot rule out the possibility that the well known action of the *Streptomyces* antibiotics on protein synthesis is producing the effects on COCB stimulation. Thus, initial irritation followed by destruction of *selected* hair cells and afferents could be responsible for the type of results observed. One has to ask, then, if the pharmacological action on the efferent synapse is responsible for the selective toxicity or if it is only coincidentally related. If this pharmacological synaptic activity of neomycin is responsible, block of efferent activity would be *followed by* destruction of the outer hair cells and then those afferents synapsing with them. Kohonen (1965) has demonstrated that the degree of efferent innervation correlated with the pattern of hair cell degeneration produced by neomycin and kanamycin, indicating that the activity of these drugs on the cochlear efferents could be responsible for their ototoxicity.

It is also possible that neomycin is exerting a pharmacological action at the efferent synapse *and* acting on protein synthesis. If this is the case, to what extent the pharmacological action plays a role in the development of ototoxicity is highly conjectural.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr Bernard Powers and Mr Clarence Foster for their excellent technical assistance.

ZUSAMMENFASSUNG

Die Wirkung des Neomycin auf die mikroskopische Morphologie der Cochlea wurde mit seinem Einfluss auf die Stimulierung des kontralateralen olivocochlearen Bundels (KOCB) von Katzen verglichen, die täglich subkutane Injektionen von Neomycin (100 mg/Kg) für die Dauer von 0, 4, 6, 7, 10 und 12 Tagen erhielten. Eine Potenzierung des KOCB-Stimulierungseffekts ist die erste wahrnehmbare Auswirkung. Sie ist am stärksten nach 6 oder 7 Tagen der Neomycinbehandlung. Danach erfolgt eine Blockierung des KOCB-Stimulierungseffekts, die ihr Maximum am 10. bis 12. Tage der Neomycinbehandlung erreicht. Die Cochlea zeigt in diesen Stadien nur minimale morphologische Schäden, womit deutlich wird, dass die Potenzierung und Blockade des KOCB-Stimulierungseffekts auf pharmakologischen Einwirkungen an der efferierenden Synapse beruht, die auftreten, bevor sich ausgeprägte Zeichen der Ototoxizität nachweisen lassen. Es wird angenommen, dass die beschriebene, pharmakologische Aktivität des Neomycin auf synaptische Funktionen für die Genese der Ototoxizität von Bedeutung ist.

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Acta Otolaryng 76 136–148 1973

THE HEALING OF THE EXTERNAL COCHLEAR WALL IN THE GUINEA PIG AFTER MECHANICAL INJURY

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(Received January 20, 1973)

Abstract The bony and vascular repair process of the cochlea was studied after mechanical lesions on the external cochlear wall in the guinea pig. Bony healing of the bulla and the cochlear wall occurred surprisingly rapidly Intracochlear hemorrhage appeared to be controlled by reflex closure of the supplying vessels. In those cases where the external membranous wall was dented it soon assumed its normal position. In some cases there was labyrinthitis or fibrosis of the intracochlear structures. However most frequently all cochlear elements appeared to heal except for a gap remaining in the vascular pattern of the external wall and an increasing degeneration of the organ of Corti with time lapse postoperatively The present investigation reveals a surprising ability of the guinea pig cochlea to heal after mechanical injury

Technical, experimental and clinical research have during recent decades led to advances in middle ear surgery Besides further developments in this field the future of otologic surgery will include conservative or reconstructive surgery of the inner ear In connection with the treatment of Menière's disease, inner ear surgery is being performed employing mechanical- ultrasound- laser and cryotechniques. However a great deal of information regarding inner ear structure and function is necessary for development of appropriate clinical techniques and therapy

The clinical entity of hearing impairment and particularly of sudden deafness is often presumed to be caused by vascular accidents such as hemorrhages, stasis, spasm, embolus and thrombus formation. These etiological mechanisms have for obvious reasons rarely been confirmed by histopathological examinations. Instead attempts have been made to imitate these vascular accidents and the healing course after traumatic injuries in animal experiments by mechanical cochlear lesions (Lurie, 1942 Borghesan 1957 Kristensen 1959 Duvall, 1968) by the influence of cold (Beck 1959) by mechanical vascular occlusions (Perlman, 1952, 1966 Kimura & Perlman 1956 1958 Perlman & Kimura, 1957 Perlman et al., 1959 Koide et al 1959 Griffith 1961 Lawrence, 1966, Bernstein & Silverstein, 1966 Morizono & Johnstone, 1968), and by embolic vascular occlusions (Alford et al., 1965 Suga et al., 1970)

The results of these experiments have also been used in attempts to elucidate the important question of the oxygen supply to the organ of Corti.

The aim of the present investigation was to study the bony and in particular the vascular healing in the guinea pig cochlea after well defined mechanical injuries.

MATERIAL AND METHOD

Forty-two adult guinea pigs underwent surgery under general anesthesia. A ventral ap-

This investigation was supported by grants from the Swedish Medical Research Council No. B 72 12X 2885-03



Fig. 1. The contralateral unoperated cochlea of a guinea pig demonstrating the site of mechanical destruction in the external wall. *RW* = round window



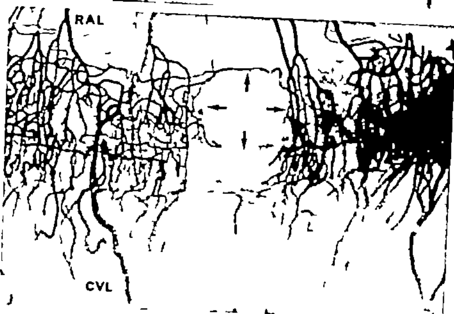
Fig. 2. Operated guinea pig cochlea demonstrating the assessment of the operated region by a tangential apicobasal section removing the destructed area and demonstrating the modiolus with a gap in the radiating arterioles corresponding to the destructed area. *VAC* = vena aqueductus cochleae.

proach to the bulla was employed. A hole was drilled in the bulla, sufficiently large to permit visualization of the basal cochlear turn. Halfway between the round window and the bulla wall, the bony cochlear capsule in the basal turn was thinned by drilling (Fig. 1). A small hole was made through the bone and under high magnification a small lesion (300–500 μm) was produced in the spiral ligament at the level of the scala media. Generally the lesion also affected the scala vestibuli and the scala tympani in the basal turn. The holes through the cochlea and the bulla were left open and the skin sutured. The contralateral ear was left intact as a control. At various intervals between one and 261 days (Table I) after the operation the animals were sacrificed and injected with a contrast medium, Berlin blue, according to a method described previously (Axelsson, 1968, 1971). The subsequent preparation steps consisted of fixation, decalcification and studies under the stereomicroscope. The operated region and surrounding areas were first assessed in one piece by a tangential large section before further dissection (Fig. 2). Photographic documentation of the findings was achieved with the aid of the photomicroscope. (Orthoplan, Leitz and Tessovar Zeiss). Changes in cellular morphology were studied with phase contrast microscopy.

Table I. *Interval between cochlear destruction and subsequent examination*

Interval (days)	No. of animals
10	7
11 20	7
21 30	5
31 50	6
51–60	4
61 100	6
100	7


RAL
CVL


RAL
CVL


RAL
CVL

In 9 animals the injection was insufficient for estimation of the vascular healing. However information was gained on the bony healing process, hair cell damage etc.

RESULT

The hole in the bulla

There was a surprisingly fast healing of the operated hole in the bulla. In those 14 animals examined within 20 days of operation, 7 holes were still open, 2 partly healed and 5 completely healed. Five of these 14 animals were examined within 3 days after operation all had open operation-holes. In a group of animals examined between 15 and 25 days after operation only one out of 8 animals had an open operation hole. After 18 days only 5 cochleas had a partly healed or open hole, 2 of these suffered from purulent otitis media.

In 11 cochleas the middle ear contained more or less purulent material. In 7 of these cochleas the middle ear changes were accompanied by labyrinthitic changes of the cochlea. In the remaining 4 the labyrinthine fluids were macroscopically unaffected.

Periosteum vessels. In well-injected preparations these vessels were easily demonstrated. In cases with labyrinthitis and fibrosis in the cochlea the periosteum vessels were remarkably dense in the operated area, usually with one big vessel penetrating into the cochlea. In the early experiments the lesion was produced close to the bulla wall. The result was a particularly rich vascular supply in the region of the injury with fibrotic adhesions between the cochlea and the bulla periosteum.

Fig. 3. External cochlear wall with operated region. Short postoperative injection interval. Above: 2 days, Middle and Below: 3 days postoperatively. Note the gap in the vascular pattern. *RAL* radiating arterioles of the scala vestibuli. *CVL* collecting venules of the scala tympani. Arrows indicate the border of operated region.

The hole in the bony cochlear capsula

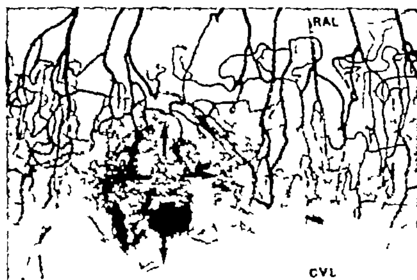
The bony lesion in the basal turn first healed by soft tissue covering the hole and later by complete bony healing. Fibrotic healing first appeared by the 10th to 15th day and complete bony healing around the 50th day after operation (Figs. 3–5). In 3 cochleas, 86, 140 and 218 days after operation, the operated lesion had not healed and there was still a hole through the bony external wall. In 2 of these cochleas clear labyrinthine fluids and a "normal" vascular repair process was found, in the third there were pronounced fibrotic changes of the whole cochlea.

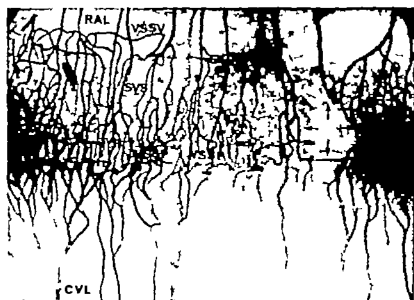
The spiral ligament in the operation region

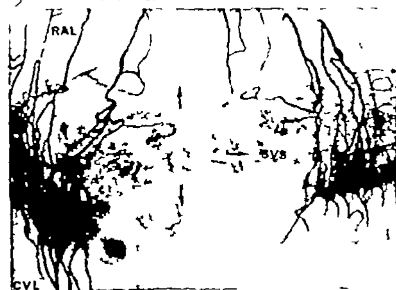
At the operation the spiral ligament was either penetrated by the sharp dissection instrument or pushed forward into the scala media. The most common finding in the post mortem studies was a combination of a central hole through the spiral ligament and an inwards dislocation of its surrounding parts. Repositioning of the spiral ligament was first observed 15 days after operation. Fifty days after operation almost all cochleas demonstrated a spiral ligament at the normal position on the inside of the cochlear capsula. Apparently and as expected the most pronounced adhesions and scars occurred between the pushed-in spiral ligament and the external parts of the basilar membrane i.e. in the region of the external spiral sulcus and the zona pectinata. Consequently there were always difficulties in dissecting the spiral lamina free from the external wall, which is necessary for the photographic documentation (Fig. 10).

Intralabyrinthine fluids

Immediately after the mechanical injury blood can be seen "curling" into the intralabyrinthine fluids. Surprisingly however there was very little evidence of hemorrhage in the cochlear fluids even in animals sacrificed within the 3 days of surgery. Moreover blood corpuscles were often demonstrated on


RAL
CVL


RAL
VSSV
SVS
CVL


RAL
SVS
CVL

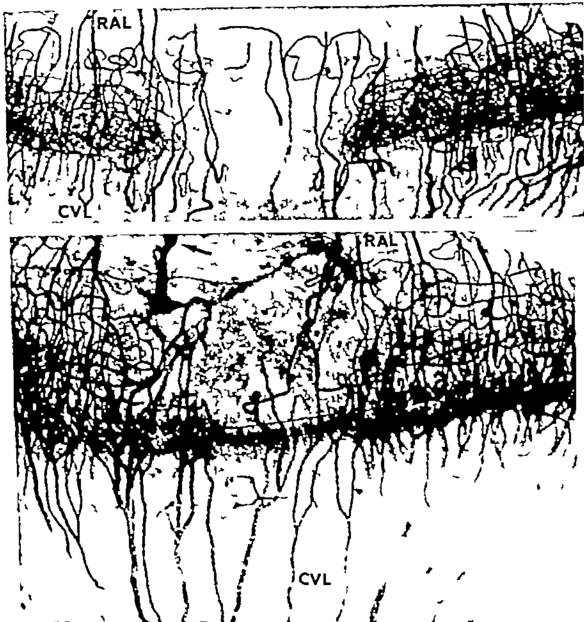


Fig. 5. External cochlear wall with operated region. Long postoperative injection interval. Above: 79 days. Below: 142 days postoperatively. Note the complete bony healing and the remaining gap in the vascular system. *RAL*, radiating arterioles of the scala vestibuli; *SVS*, stria vascularis; *CVL*, collecting venules of the scala tympani. *Arrow*: large pathological periosteum vessel.

Fig. 4. External cochlear wall with operated region. Moderate postoperative injection interval. Above: 10 days. Middle: 15 days. Below: 38 days postoperatively. Note the bony healing in the destroyed area and the gap in the vascular pattern. *RAL*, radiating arterioles of the scala vestibuli; *VSSV*, the vessel of the scala vestibuli; *SVS*, stria vascularis; *VSSP*, the vessel of the spiral prominence; *CVL*, collecting venules of the scala tympani. Arrows indicate the border of the operated region.



Fig 6 Apicobasal section of basal cochlear turn 50 days postoperatively. Fibrosis of all scalas as a result of labyrinthitis. *SMA* spiral modiolar artery. *SMV* spiral modiolar vein. *SV* scala vestibuli. *ST* scala tympani. *LS* lamina spiralis.



Fig. 7 The "glomeruli" the spring-coil appearing proximal parts of the radiating arterioles in the modiolus. Corresponding to the operated region there is always a gap in the arterioles (*arrow*). *CVL* collecting venule of the second turn.

the inner ear membranes, e.g. the vestibular membrane and particularly in the region of the external spiral sulcus. In 9 cochleas at different times after the operation there was a discrete precipitate in the intralabyrinthine fluids of the basal turn as evidence of some labyrinthitis or remaining blood and cellular debris. In 7 cochleas there was more or less organized fibrotic changes in the basal turn as evidence of a more severe labyrinthitis with secondary changes (Fig. 6). The earliest of these was demonstrated 22 days after operation.

Modiolus

In all guinea pigs there was a consistent change of the radiating arterioles in the modiolus. In the region of the "glomeruli" the radiating arterioles corresponding to the destroyed area were not filled by contrast. This included a lack of filling in the region of corresponding external wall (Figs. 7 and 8). This gap in the radiating arterioles was one of the most regular findings after the mechanical lesion. It was consistently found only in that region of the cochlea associated with destruction.

Spiral lamina basilar membrane and organ of Corti

The only regular change in these structures was a more or less pronounced hair cell degeneration, most prominent in the outer hair cells. The degeneration was always segmentally limited to a fairly small sector opposite to the site of destruction. The degeneration appeared to be more pronounced and more widespread, the longer was the interval from the operation (Figs. 9–11). Some degenerated material (debris, blood corpuscles) was always observed on the basilar membrane, on the spiral limbus and the vestibular membrane.

The vessels of the spiral lamina generally appeared normal. In those cases with secondary fibrotic adhesions between the spiral ligament and the basilar membrane short spiral defects of the vessel of the basilar membrane could be demonstrated (Fig. 10 below). It was not possible to judge whether these defects



Fig. 6. Radiating arterioles of the scala vestibuli immediately peripherally to the "glomeruli" There is a gap (*arrow*) in the radiating arterioles corresponding to the operated region. *RAL*, radiating arterioles running apico-basally· *CVL*, a collecting venule of the scala vestibuli running in a spiral direction centrally

were the result of scarring or of the difficult post-mortem preparation of this region.

Vascular structures of the external wall

The normally appearing and regularly occurring vessels of the external wall are

Scala vestibuli

Radiating arterioles
Collecting venules
The vessel of the scala vestibuli
The vessel at the vestibular membrane
A capillary net above the vestibular membrane

Scala media

Stria vascularis
The vessel of the spiral prominence
Arterio-venous anastomoses

Scala tympani

Collecting venules
The venules at the basilar membrane.

Since the lesion in general was most prominent in the scala media, these vessels also demonstrated the most pronounced changes in the destroyed region. However the combination of the above-described unfilled radiating arterioles, the destroyed area in the scala media and the subsequently unfilled collecting venules of the scala tympani resulted in the typical finding of a narrow apicobasal gap in the vascular pattern with the destroyed area in the centre (Figs. 3–5) This appearance was consistently observed both in cochleas with a fresh injury and in animals with a completely healed hole in the bony capsule and a restored position of the spiral ligament. In some cochleas sparse vessels could be seen crossing the operated region mainly in an apicobasal direction in this "vascular gap" The stria vascularis was never observed to resume any vascularisation in the operated area. In no case within 261 days after destruction was there any complete vascular repair The vascular pattern in nearby or more distant located areas of the cochlea was always normal.

DISCUSSION

As evidenced from the present material the following events appear to occur after mechanical lesions of the external cochlear wall. First, there must be a considerable initial haemorrhage from the dense vascular net in the scalae, particularly in the scala media. Apparently this haemorrhage is soon controlled by "a reflex" closure of the supplying radiating arterioles at the level of the so called "glomeruli" in the modiolus. The blood and debris in the intralabyrinthine fluids is generally absorbed, probably in the external spiral sulcus. In some cases the end-result is a fibrosis as a consequence of a labyrinthitis, or of an organization of the material in the intralabyrinthine fluids. This fibrosis seems to be more frequent when the lesion was close to the bulla wall with resultant periosteal scarring. The hole on the cochlea is early covered by soft tissue. In most cases the repair is astonishingly rapid and is most prominent in the bony elements of the bulla and the external cochlear wall. If the spiral ligament is pushed into the endolymph initially it later resumes its normal position. However the vascular elements of the external wall do not recover a normal pattern




OHC


SBM



Fig. 10. Large sections of the spiral lamina opposite to the operated region. Above: 62 days, Below 71 days postoperatively. According to adhesions between the pushed in external wall and the spiral lamina the direction of the area corresponding to the destruction is often difficult and the vessel of the basilar membrane (*VSBM*) and the vessel of the tympanic lip (*VSTL*) destroyed by the direction (Above: arrow). *OHC* outer hair cells, *IHC* inner hair cells, *CVL* collecting venules of the external wall. Note the spotty and pronounced degeneration of the organ of Corti.



Fig 11 Spiral lamina phase contrast, high magnification. 261 days postoperatively In spite of very severe degeneration of the hair cells the vessel of the basilar membrane (*V SBM*) and the vessel of the tympanic lip (*V STL*) appear normally contrast-injected. *OHC* outer hair cells, *IHC* inner hair cells.

at least not within months after the injury Instead there is a narrow avascular segment originating in the "glomeruli" and extending over the external wall in all scalae. In the spiral lamina the only regularly remaining injury afflicts the organ of Corti on a rather narrow segment, however apparently increasing with the postoperative time These findings are in agreement with the sparse information obtained by other authors (Kristensen 1959).

From the above findings certain conclusions may be allowed. The small segmentally restricted injury of the organ of Corti observed in the basal turn is consistent with so called radial flow theory of endolymph. The large injury produced in the present experiments otherwise would have been expected to cause much more widespread lesion in a spiral direction. This finding is in agreement with other previous experimental investigations concerning the same question (Alford et al., 1965 Duvall et al., 1969). The seemingly reflex-induced closure of the radiating arterioles at the level of the "glomeruli" may indicate one function of these unusual structures. The "vascular spring-coils" in the modiolus consist of tightly coiled parts of the radiating arterioles. They are surrounded by circulate smooth muscle cells and a longitudinal "elastica interna" internally (Schwalbe 1887) or by sparse smooth muscle cells (Smith, 1951) The vessels are surrounded by abundant autonomic nerve fibers (Mitsebeck & Mootz, 1966 Terayama et al. 1966). The curious and specific arrangement of the "vascular spring-coils" speaks in favour of a particular and specific function (Axelsson, 1968). The existence of smooth muscles might indicate a regulating function active or passive of events in more peripheral capillary regions.

The rich vascular supply to the external wall was always damaged without resuming a normal vascular pattern. This finding warrants great caution in drawing conclusions from experiments where electrodes and other measuring devices are introduced in the cochlea of the living guinea pig. On the other hand the frequent finding of a steady state during such experiments may be explained by an arterial obstruction of the "glomeruli" presumed in the present investigation If the afflicted vessel gave rise to considerable hemorrhage an equilibrium state would probably not occur

In regards to future attempts of inner ear surgery of interest is the finding that the injury of the organ of Corti is limited to a small sector and that surgical attempts may be accomplished without the risk of deleterious bleeding. Furthermore a result of the present investigation is the finding that even after pronounced lesions to the cochlear and paracochlear structures, all may heal except the sensory cells and the vascular structures. The finding of a limited area of scarring in these structures does, however not admit conclusions on the final function of a mechanically damaged hearing organ. Until cells with closely massed microvilli suggestive of light cells and smooth cells with sparse, short microvilli suggestive of dark cells, both of which are all arranged irregularly and form the so-called rugose structure The entire intermediate portion is concerned with promi-

functional measurements have been made little can be said about the risk of surgically exposing the inner ear However the inner ear function after experimental lesions must be measured by other devices than intracochlear electrodes (Suga et al., 1970) which obviously may produce a considerable trauma to the cochlea. As an alternative electrical potentials apparently may be determined by extracochlear devices (Alford et al., 1965) and possibly by other techniques e g. hearing test ing after conditioning (Andersson & Wedenberg, 1965 Andersson et al. 1968 Stebbins, 1972, Moody 1972).

The sparse experiments with mechanical injuries to the cochlea seem to have resulted in a high frequency of intracochlear fibrosis as an end-result. The present investigation demonstrated that if a delicate technique is applied, small lesions in a majority of cases need not be followed by fibrosis. It is also interesting that when the experimental lesions were followed by middle ear suppuration this did not very often cause a labyrinthitis nor did the presence of a remaining open hole without the bony closure probably due to the fact that the mechanical lesions were very limited in a spiral direction. All these findings indicate an interesting and surprising ability of the cochlea to defend itself" against destructive injuries produced on the lateral cochlear wall and to limit intra-cochlear damage.

On the basis of the cochlear vascular pattern and the above experiments it may be suggested that a small lesion which is experimentally induced or represents the result of a clinical pathological condition might be compensated for by the rich vascular anastomosing possibilities. Contrarily a large lesion representing a serious risk for destruction of the sensory organ might be limited by reflex closure of the vessels in the afflicted segment of the cochlea.

ZUSAMMENFASSUNG

An der äusseren Cochlearwand eines Meerschweinchens wurde der Heilungsvorgang des Knochens und Zellgewebes nach einer mechanischen Schädigung beobachtet. Die Knochenheilung der Bulla und Cochlearwand erfolgte überraschend schnell. Es erschien als ob intracochleare Blutungen an einem Schliessungsreflex der Versorgungsblutgefässe unter Kontrolle gehalten wurden. In Fällen bei welchen die äussere membranöse Wand eingedrückt war zeigte es sich, dass diese später wieder ihre normale Position einnahm. In einzelnen Fällen konnte man Labyrinthitis oder Fibrosis der intracochlearen Struktur beobachten. Es zeigte sich im allgemeinen, dass alle Cochlearelemente schnell zur Heilung neigen wenn man dabei die Regenerationslücke des Blutgefäss-Systems und die zeitbedingte postoperative Degeneration des Cortischen Organs mitbeachtet. Das Resultat der vorgelegten Untersuchungen zeigt ein überraschend gutes Heilungsvermögen der Cochlea des Meerschweinchens nach einer mechanischen Schädigung.

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Acta Otolaryng 76 149–156, 1973

OBSERVATION OF LUMINAL SURFACE OF THE ENDOLYMPHATIC SAC IN GUINEA PIG

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(Received November 10, 1972)

Abstract By three-dimensional observation under the scanning electron microscope of the luminal surface structures of the endolymphatic sac, the following results were obtained.

1 Based on surface morphology the endolymphatic sac may be divided into 3 portions; the proximal, intermediate and distal.

2 The proximal portion consists of flat surface, polygonal duct-type cells with short microvilli.

3 The intermediate portion is composed of 2 kinds of cells; cells with closely massed, long microvilli and the other smooth surfaced cells with short microvilli. These cells are arranged irregularly forming papillae and crypts.

4 The distal portion has 2 kinds of cells as observed in the intermediate portion. In this portion, however the number and length of microvilli are less and cells are rather sparse At the extreme end, the cells are gradually transformed into duct-type cells.

5 Judging from morphological observations of the surface structures, it is apparent, but not proved, that the intermediate portion is functionally most active.

Lundquist et al. in 1964 first made the electron microscopic observation of the endolymphatic duct and sac in normal guinea pig. Since then there has appeared one additional report concerning the ultrastructure of the endolymphatic duct and sac (Lundquist, 1965). The electron microscopic study by Lundquist in which he employed serial sections of endolymphatic duct and sac is the most excellent method for the investigation of the fine intra and intercellular features and the ultrastructure of the subepithelial tissues, but it is not quite adequate for obtaining three-dimensional profiles of these structures as a whole

With the scanning electron microscope, however it is possible to observe the surface structures of whole tissues in three dimensions and thus provide more suitable three-dimensional views of the fine morphologic features of the endolymphatic duct and sac.

In this report we attempt to present some findings of the surface structures of the normal endolymphatic sac in the guinea pig using the scanning electron microscope.

MATERIAL AND METHOD

The animals used were young adult guinea pigs of both sexes, weighing 250–350 g with normal Preyer reflex. After decapitation, the temporal bone was removed as soon as possible without disturbing the endolymphatic sac and the sigmoid sinus. The stapes and the round window membrane were removed and the sinus was opened under a surgical microscope. Next, by flushing the fixative (1% osmic acid and 2.5% glutaraldehyde buffered with cold phosphate solution, pH 7 4) into the vestibule and the sigmoid sinus with a fine glass pipette, the entire specimen was steeped in cold fixative for 3 hours. After fixation, the endolymphatic sac was dissected



Fig 1 Luminal surface of the endolymphatic sac after dissection of the vestibular aqueduct, showing the superior (*CsS*) and posterior (*CsP*) semicircular canals, and the endolymphatic duct and sac (*SE*) which are stained dark with osmic acid. Endolymphatic duct enters into the vestibule from the posterior medial site of common crus (*CC*). Temporal preparation (*left*).

with fine forceps and dental burrs submerged in a buffered solution in order to divide the sac into two halves. Then the graded ethanol dehydration and final dehydration with isoamyl acetate were carried out The specimens were dried by a vacuum evaporator The dried specimens were given a double coating of carbon and gold and these were observed with scanning electron microscope, JSM U 3 (Japan Electron Optics Lab)

RESULT

It seems appropriate to divide the endolymphatic sac into 3 different parts, the proximal intermediate and distal portion, as Lundquist described

1 *Proximal portion*

The proximal portion of the sac is a part which widens gradually and is approximately

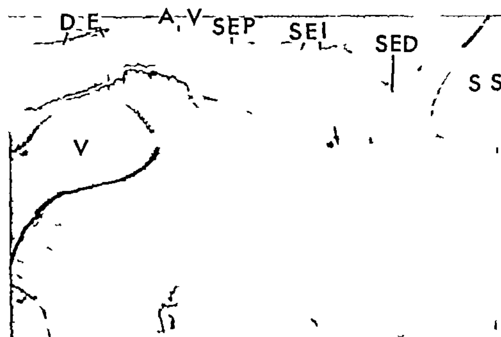


Fig 2 A slightly oblique sagittal section of the temporal bone. The endolymphatic duct (*DE*) begins in the vestibule (*V*). A portion of the endolymphatic sac lies within the vestibular aqueduct (*AV*) and a portion of it is closely in contact with the sigmoid sinus (*SS*). The endolymphatic sac can be divided into three portions: the proximal (*SEP*), intermediate (*SEI*) and distal (*SED*).



Fig 3 The major part of the proximal portion consists of polygonal, smooth surfaced, flat duct type cells with short sparse microvilli. ×4 000.

0.5 mm long In the first part of this portion, near the duct, there can be seen epithelial cells which have a flat and polygonal surface and all of which have a distinct cell boundary Among these duct-type cells, there can be observed, on occasions, some epithelial cells with a different appearance having numerous, moderately long microvilli, some of these microvilli are flat tipped. The cells of this portion of the sac undergo gradual transition from the duct-like type to a convex type as they approach the adjoining intermediate portion. The numbers and size of microvilli increase gradually

2. *Intermediate portion*

The intermediate portion partly extrudes out of the vestibular aqueduct It is about 2 mm

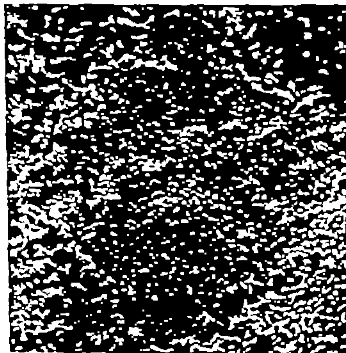


Fig 4 A greater magnification of the duct-type cell. ×7 600

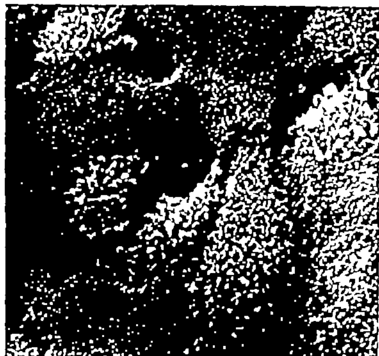


Fig. 5. Among the duct-type cells, there can be observed on occasion, some epithelial cells of different features having many moderately long microvilli, some having flat tips. ×2 850

in length and 1 mm in width. It is considered to be the main part of the endolymphatic sac. At this portion the morphology and size of the epithelium vary considerably and the region is composed of the epithelium with densely massed long microvilli forming a rosette and the smooth epithelium with a few short microvilli. Epithelial cells in this region are irregularly arranged forming papillae and crypts, and present a structure cor



Fig. 6. In the intermediate portion there can be seen two different kinds of cells, cells with densely massed long microvilli suggestive of light cells and smooth-surfaced cells with sparse, short microvilli suggestive of dark cells. 3 000.

responding to the so-called rugose portion, which can readily be distinguished from the proximal portion.

3 *Distal portion*

Distal portion is in close contact with the sigmoid sinus. It is about 0.5–1 mm in length, its luminal surface is rather smooth with no papillae or crypts. The epithelium apparently has features similar to those of epithelium in the intermediate portion. However the cells in the distal portion, compared with those in the intermediate have a rather smooth surface and their microvilli are shorter and fewer In the vicinity of the extreme end, the profile of the cells approaches that of flat cells which are observable in the proximal portion having a few short microvilli. These terminal cells are surrounded by the connective tissue of dura mater and their number also decreases.



Fig. 7 A greater magnification of the epithelial cell with closely massed long microvilli forming rosettes. It appears to be a light cell. × 7 450.

COMMENT

There are many reports (Guild 1927 Bast & Anson, 1949· Allen, 1964 Engström & Hjorth, 1950· Lundquist et al., 1964 Lundquist, 1965 and others) concerning the endolymphatic duct and sac. As far as we know however there seem to be no published reports dealing with the surface ultrastructure of the lumen

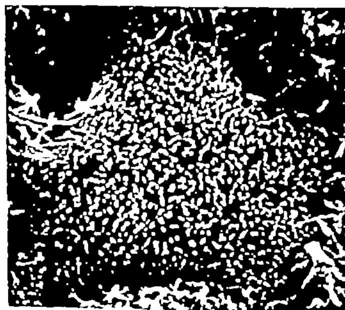


Fig. 8. A greater magnification of the epithelial cell suggestive of dark cell with a few short microvilli. 8 000.

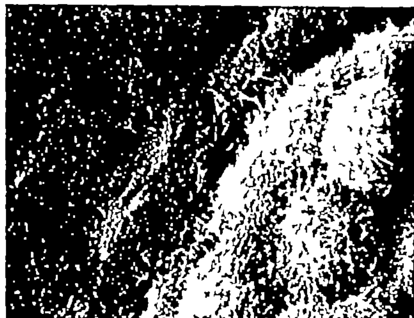
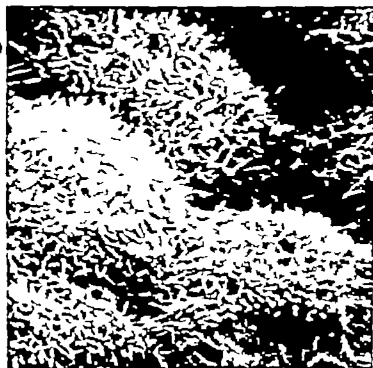


Fig 9 In the intermediate portion, epithelial cells are irregularly arranged forming papilla (lower corner), and shows a structure corresponding to the so-called rugose portion, which may be readily distinguished from the proximal portion seen at the left of the photograph. × 2 800.

of the endolymphatic sac observed with scanning electron microscope For this reason we carried out the scanning electron microscopic observations of the luminal surface of the endolymphatic sac in guinea pigs.

The proximal portion is an extension of the endolymphatic duct to the intermediate portion where the activity is said to be highest in the endolymphatic sac. The surface structures of these smooth-surfaced epithelial cells with short microvilli suggest a low surface activity

At the intermediate portion there can be seen two different kinds of cells; namely,



nent surface activity. The portion of human endolymphatic sac which Bast & Anson (1949), Anson (1969) call the rugose portion seems to correspond to the combination of the proximal portion and the major part of intermediate portion of the endolymphatic sac of guinea pig.

The cells of the distal portion show characteristic features similar to those of two kinds of cells observed in the intermediate portion. However, these two kinds of cells have a rather flat surface and the number and length of their microvilli are less, which seems to suggest that their surface activity also must be less. They have no evidence of papillae. At the extreme end of the distal portion, the cells present a profile similar to that of duct type cell, and their surface as a whole appears wavy undoubtedly because of being in a close contact with fibrils of the dura mater. From these findings it may be safe to say that the activity of the endolymphatic sac is less in the direction of the terminal end.

To comprehend the organization of such minute surface structures in three dimensions would be important for studies of physiological functions of the endolymphatic sac tissues.

ZUSAMMENFASSUNG

Die dreidimensionale Beobachtung der Oberflächenstruktur des Raumes des Saccus endolymphaticus mit dem Rasterelektronenmikroskop kam zu folgenden Ergebnissen.

1. Der Saccus endolymphaticus wird aufgrund der äusseren Form in 3 Teile, d. h. den proximalen, den intermediären und den distalen, eingeteilt.

2. Der proximale Teil besteht aus den flachen glatten und polygonalen Ductus-Typ-Zellen mit kurzen Mikrovilli.

3. Der intermediäre Teil besteht aus zwei Arten von Zellen, die einen mit dicht gewachsenen, langen Mikrovilli und die anderen glatt mit kurzen Mikrovilli. Diese Zellen sind unregelmässig angeordnet und bilden Papillen und Krypten.

4. Der distale Teil besteht aus den gleichen Zellen wie der intermediäre. Aber in diesem Teil sind die Mikrovilli gering und kurz. Auch die Anzahl der Zellen ist klein. Am äussersten Ende gehen die Zellen allmählich in die Duktus-Typ-Zellen über.

5. Nach der morphologischen Beobachtung der Oberflächenstruktur scheint der intermediäre Teil funktionell der aktivste zu sein.

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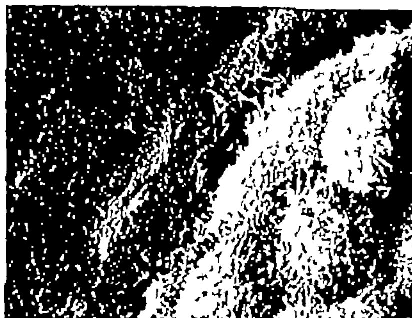


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At the intermediate portion there can be seen two different kinds of cells named

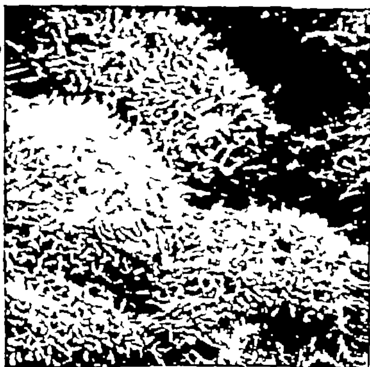


Fig. 10. In the distal portion, the epithelial cells have profiles similar to those of cells in the intermediate portion. However, the cells in this portion, compared with those in the intermediate portion, have a smooth surface and their microvilli are shorter and less numerous. × 6 800.

Table I *Streptomycin concentrations (µg/ml) after intrathecal injection of 200 mg/kg SM sulphate*

Number of animals	Time of sampling (minutes post-injection)					
	1	5	10	15	30	60
Perilymph	10 000	7 000	5 400	1 000	600	250
Serum	150	177	240	460	320	140
Lymph nodes	*240*	*310*	*350*	*510*	83	36
Heart	29	9 7	16	250	88	11
Liver	0	5	0	11	13	0
Muscle	19	9	22.5	105	160	20

cal lymph nodes, perilymph from the round window membrane and blood. The microbiological assays were carried out with serum and organ homogenates. The latter were prepared with organs removed under sterile conditions from the killed test animals at given intervals after streptomycin administration and suspensions prepared in 0.9% NaCl solution (final dilution 1 10, except for the cervical lymph nodes where it was 1 20)

Streptomycin levels in the serum and organ homogenates were determined by the vertical diffusion test (VDT) after Schmiedel (1958) as modified by Wagner et al. (1963), using a strain of *M smegmatis* as the test organism.

The streptomycin levels in the perilymph were determined by the filter disc test using *B subtilis* ATCC 6633 as the test organism (for further details of these methods, see Wagner et al. 1971). Generally one animal was used for each concentration assay exceptions are the 5 and 10 min values, which are the means of the results from 3 animals.

Second series. Injection of colloidal solution.

Administration of preparation

Intrathecal injection of colloidal gold solution (^{198}Au, mean particle diameter 50 Å, spec. act 3 1 mCi mg Au) The animals were killed at the given times. Cervical lymph nodes, heart muscle liver mucosa from the epipharyngeal region and blood were sampled for measurement of the activity of the gold isotope. The total activity in the syringe prior to injection was measured by gamma scintillation counter in defined geometry After the injection the residual activity in the syringe was measured. Activity in the blood and organs was measured in the borehole of the scintillation crystal. A conversion factor between the two instruments and the radioactivity decay were taken into account in the calculation of all results. Generally three animals were used for each activity assay and killed after 10, 25 45 and 60 min. For control the same radioactive gold (^{198}Au, ⌀ 50 Å) was administered intravenously to rats. Five animals were used for each activity assay

RESULT

First series

The concentration of the antibiotic in serum lymph nodes, heart muscle liver and diaphragm was determined 1 5 10 15 30 and 60 min after the intrathecal injection of 200 mg/kg streptomycin sulphate into guinea pigs (Table I). At 1 min the highest streptomycin concentration was found in the perilymph (10 000 µg/ml) followed by the streptomycin concentration in the cervical lymph node homogenate (240 µg/ml) The other concentration levels all showed a rise early in the observation period but the maxima always occurred later than in the perilymph curve and in lymph nodes. The descending sections of the elimination curves all have the same slopes.

The elimination of streptomycin sulphate from the perilymph is much delayed and it is evident that the perilymph level is constantly over the blood serum level up to 60 min after the injection. Remarkably the cervical lymph node concentration remains until 15 min after the injection above that of the blood. Contrasting conspicuously with other body tissues the liver contained less streptomycin than did muscle. In the comparative trial where 200 mg/kg streptomycin sulphate was given to guinea pigs intramuscularly there

Acta Otolaryng 76: 156–161 1973

QUANTITATIVE STUDIES ON THE DRAINAGE OF THE CEREBROSPINAL FLUID INTO THE LYMPHATIC SYSTEM

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(Received December 15 1972)

Abstract Molecular solutions and colloidal particles, when introduced into the CSF flow out very rapidly to the periphery by the direct lymphatic connections and attain higher concentrations in unit time in the deep cervical lymph nodes than would be possible via the bloodstream. The demonstrated CSF-lymphatic outflow pathway represents a direct communication between brain and lymphoid tissue. One may therefore presume that the deep cervical lymph nodes provide the first site of filtration for degradation products in the CSF

Recent light and electron microscopic studies had shown that the cerebrospinal fluid (CSF) has numerous communications with the lymphatic system in the head and neck region in addition to its known outflow pathways (absorption by the ependyma absorption into the meningeal veins, absorption by the arachnoid villi and reabsorption by the choroidplexus). The morphological findings, though permitting topographical localization of the additional outflow pathways, did not allow any conclusions to be drawn as to the rate of CSF clearance via the lymphatic route It also remained uncertain whether substances dissolved in the CSF reached the lymphatic system in the head–neck region faster by way of the blood or by the direct lymphatic pathway Since to our knowledge no results have yet been published on any study of this subject, and as the relevant data might be of clinical and even therapeutic value, we heve investigated the kinetics of the CSF-drainage via the lymphatic route in a model experiment with guinea pigs, using molecular solutions and isotopes.

MATERIAL AND METHOD

Animals Guinea pigs (heterozygous stock, body weight 270–460 g)

Experimental Design

First series Injection of a molecular solution of streptomycin sulphate determination of concentration by microbiological assay

Administration of preparation

(*a*) 200 mg streptomycin sulphate in physiological saline per kg body weight was administered by slow intrathecal injection into the cerebello-medullary cistern. The injected volume was about 150 μl. The injection was made with great care and without the application of pressure (for method of pressureless administration see Arnold et al. 1972)

(*b*) For purposes of comparison other guinea pigs received 200 mg streptomycin sulphate/kg body weight by deep intragluteal injection.

Microbiological assay

Immediately after decapitation of the animals, samples were taken of muscle tissue (diaphragm) liver heart muscle, deep cervi-

Table I *Streptomycin concentrations (µg/ml) after intrathecal injection of 200 mg/kg SM sulphate*

Number of animals	Time of sampling (minutes post-injection)					
	1	5	10	15	30	60
Perilymph	10 000	7 000	5 400	1 000	600	250
Serum	150	177	240	460	320	140
Lymph nodes	240	310	350	310	98	36
Heart	29	9 7	16	250	88	11
Liver	0	5	0	11	13	0
Muscle	19	9	22.5	105	160	20

cal lymph nodes, perilymph from the round window membrane and blood. The microbiological assays were carried out with serum and organ homogenates. The latter were prepared with organs removed under sterile conditions from the killed test animals at given intervals after streptomycin administration and suspensions prepared in 0.9% NaCl solution (final dilution 1 10, except for the cervical lymph nodes where it was 1 20).

Streptomycin levels in the serum and organ homogenates were determined by the vertical diffusion test (VDT) after Schmiedel (1958), as modified by Wagner et al. (1963), using a strain of *M smegmatis* as the test organism.

The streptomycin levels in the perilymph were determined by the filter disc test using *B subtilis* ATCC 6633 as the test organism (for further details of these methods, see Wagner et al. 1971). Generally one animal was used for each concentration assay exceptions are the 5 and 10 min values, which are the means of the results from 3 animals.

Second series. Injection of colloidal solution.

Administration of preparation

Intrathecal injection of colloidal gold solution (^{198}Au mean particle diameter 50 Å, spec. act. 3 1 mCi mg Au) The animals were killed at the given times Cervical lymph nodes, heart muscle, liver mucosa from the epipharyngeal region and blood were sampled for measurement of the activity of the gold isotope. The total activity in the syringe prior to injection was measured by gamma scintillation counter in defined geometry After the injection the residual activity in the syringe was measured. Activity in the blood and organs was measured in the borehole of the scintillation crystal. A conversion factor between the two instruments and the radioactivity decay were taken into account in the calculation of all results. Generally three animals were used for each activity assay and killed after 10 25 45 and 60 min. For control the same radioactive gold (^{198}Au, Φ 50 Å) was administered intravenously to rats. Five animals were used for each activity assay

RESULT

First series

The concentration of the antibiotic in serum lymph nodes, heart muscle, liver and diaphragm was determined 1 5 10 15 30 and 60 min after the intrathecal injection of 200 mg/kg streptomycin sulphate into guinea pigs (Table I). At 1 min the highest streptomycin concentration was found in the perilymph (10 000 µg/ml) followed by the streptomycin concentration in the cervical lymph node homogenate (240 µg/ml) The other concentration levels all showed a rise early in the observation period but the maxima always occurred later than in the perilymph curve and in lymph nodes. The descending sections of the elimination curves all have the same slopes.

The elimination of streptomycin sulphate from the perilymph is much delayed and it is evident that the perilymph level is constantly over the blood serum level up to 60 min after the infection Remarkably the cervical lymph node concentration remains until 15 min after the injection above that of the blood Contrasting conspicuously with other body tissues the liver contained less streptomycin than did muscle. In the comparative trial where 200 mg/kg streptomycin sulphate was given to guinea pigs intramuscularly there

Table II *Concentration of streptomycin sulphate in some organs after intramuscular injection of 200 mg/kg SM sulphate (µg/ml)*

	Minutes post injection		
	15	30	60
Perilymph	1.8	2.9	4.8
Serum	260	170	160
Lymph nodes	144	121	128
Muscle	34	30	27

was no tendency for the drug to accumulate more in the cervical lymph nodes than in other tissues. The concentrations after intra muscular administration were substantially lower than after intrathecal injection also when these measurements were carried out earlier Indeed it is evident that the rate of outflow of a final maximum concentration from the cervical lymph nodes shows some delay over that from other tissues (Table II).

Second series

The intrathecal injection of radioactive gold with a particle size of 50 A led to the finding of similar concentration differences in the various tissues. In the second series, as in the first a significant accumulation of ac tivity was measurable in the cervical lymph nodes (Table III). In order to exclude the possibility that this was not merely the specific sequestration of colloidal gold by the reticulo-endothelial cells of the cervical lymphoid tissue radioactive gold (50 A) was administered intravenously to control rats. No specific accumulation of the isotope was found in the cervical lymph nodes of these control animals at up to 2 hours *post injectionem* Massive accumulation was, however found in the liver and spleen and the serum radioactivity was still three times higher than the cervical lymph node radioactivity after 30 min (Table IV) The high and enduring radioactivity measurable in the cervical lymph nodes after intrathecal injection might be explained by pinocytotic uptake of the tracer (Thorotrast was used for electron microscopic con

Table III *Activity of ¹⁹⁸Au in various body tissue (percentage of total measured activity per gram of tissue) 10 25 45 and 60 minutes after (intrathecal injection of the isotope (3 animals for eac concentration assay)*

	Minutes post-injection			
	10	25	45	60
Liver	1 41	1 71	2.25	0 58
Heart	0.012	0 0055	0.0062	0 0038
Lymph node	*8 61*	*15.05*	*14.36*	*5.86*
Epipharyngeal mucosa	0.40	0 63	1 44	0.21
Serum	0.1[illegible]	0.24	1.31	0 086

trol particle size 40 A, similar to that of ¹⁹⁸Au – 50 A) by reticulocytes lining the lymphatic *sinus marginales et medullares* and by accumulation of the colloidal particles in the intercellular spaces in the lymph node medulla (sites that are possibly reached less rapidly via the blood-stream) (Fig. 1).

DISCUSSION

Our trials with a molecular solution (streptomycin sulphate) and a colloidal solution (radioactive gold with a particle size of 50 A) show that these substances, when introduced into the CSF reach the deep cervical lymph nodes via lymphatic pathways much faster and in higher concentration than they do via

Table IV *Activity of ¹⁹⁸Au in various body tissues after intravenous injection of the isotope after 30 minutes 2 hours 24 hours and 48 hours (percentage per gram of tissue) (5 animals were used for each concentration assay)*

	Time post-injection			
	30 min	2 h	24 h	48 h
Liver	11 71	12.20	11.05	9 66
Spleen	1.89	2.04	2.23	2.39
Lymph nodes	0.20	0 29	0.27	0.30
Lung	0 42	0.30	0.19	0 70
Muscle	0 015	0.011	0 016	0 017
Heart	0.19	0 070	0 076	0 070
Serum	0.79	0.20	0.10	0 093



Fig. 1. Magnification 32 000 × of a section across the medullar sinus region of a lymph node 3 min after an intrathecal injection of 50 µl thorium-dioxide. The sinus contain many particles of the tracer which are being taken up by micropinocytosis (→) into the cytoplasm of reticulum cells (*R*). *L* – lymphocyte.

the bloodstream when injected intramuscularly. The measured tissue concentrations, too, were several times greater in the short term trial than the maximum concentrations attained via the blood. The molecular streptomycin solution produced a high lymph node

concentration due only to the high outflow rate from the CSF and this concentration equilibrated rapidly with that in other tissues as the substance moved into the bloodstream The very slow rate of decay of the colloidal gold isotope in the cervical lymphoid tissue indicated accumulation there and indeed to an extent that could not be attained even in the long term trial with an equal amount administered via the bloodstream (Stembredge et al. 1953).

Our electron microscopic studies (Arnold et al. 1972) suggest that the reason for this high activity may be accumulation of the colloidal tracer intracellularly in the cytoplasm of reticulocytes and intercellularly as deposits between the lymphocytes, sites that are apparently not accessible via the bloodstream during the short term trial for the colloidal tracer Measurements also indicate that organs in the flow through regions of the CSF lymphatic connections (perilymph, middle ear mucosa olfactoric mucosa nasal- and epipharyngeal mucosa) likewise show a considerable accumulation of the tracer It is of particular interest that the perilymph of the inner ear very rapidly accumulates the highest streptomycin concentration of all body tis-
and that this concentration falls only
,vly apparently dependent on serum con-
This not only affords further evidence of a free CSF-perilymph communica tion (Arnold & v Ilberg 1971) but also indicates in particular that the lymph circula tion of the inner ear is subject to its own laws (cf Wagner et al. 1971)

Knowledge of the existence of a CSF lymphatic communication also explains the apparently paradoxical changes in the cervical lymph node concentration after intramuscular injection of the antibiotic. The trial shows that, unlike the concentration changes in other tissues, the cervical lymph node con centration falls only very slowly an explana tion being that this lymphoid tissue accumu lates streptomycin from two sources, the bloodstream and the CSF

In consensus with many reports on mor phological findings in the literature (Key & Retzius, 1875 Hoffmann & Thiel, 1956 Brierley & Field 1948 Yoffay 1958 Arnold et al. 1972) our experiments again support the existence of CSF–lymphatic connections Evidence was also forthcoming that certain particulate materials present in the CSF reach the deep cervical lymph nodes faster via direct lymphatic outflow pathways than via the bloodstream. As our [198]Au and Thorotrast experiments show these nodes must act as an important filter station for the CSF on its way to the bloodstream The apparent rapidity of CSF transport out of the subarachnoid space by way of the lymph justifies the question whether present concepts of CSF circulation need some revision. Dissolved and undissolved substances (e g. brain-specific degradation products) can leave the CSF through the arachnoid villi (Shabo et al., 1969) the ependyma (Brightman, 1965, 1966; Feldberg & Fleischhauer 1965 Janzen, 1969) the chorioid plexus (Cserr 1971) and the lymphatics. One must consider however that penetration through the ependyma or arachnoid villi is obstructed by morphological barriers which only allow passage by an intracellular route In the region of the ependyma the morphological barriers are zonulae occludentes, subependymal astroglia, basement membranes and endothelial cells (Hager 1961). We know from the studies of Farquhar & Palade (1965) and Brightman (1966) that the movement of substances across the described cellular planes is by a transcellular route by passing the zonulae occludentes and the consequence must therefore be that the rate of passage can be determined cellularly

There are no cellular barriers in this sense on the lymphatic pathway Our investigations do not show to what extent the reticulum cells of the cervical lymph nodes regulate the passage of the fluid. But a major portion of the 50 A particles was sequestrated intracellularly in the reticuloendothelial system of the lymph node sinuses. It must also be empha-

sized that particles as large as Thorotrast, for example will penetrate as far as the intercellular spaces within lymphoid follicles.

Our findings do not allow us to exclude the possibility that CSF-lymphatic communications provide a route of access for neurotropic, viral or bacterial infections to the brain It has been demonstrated by the investigations of Orosz et al. (1957) and Czernjawska (1970) that this pathway can be utilized, even against the direction of CSF-flow Evidence was adduced that a centrifugal CSF lymphatic stream did not prevent isotopes (^{198}Au) from reaching far higher concentrations in the CSF and various topographic regions of the brain (paraventricular organs) than would be possible via the blood.

These findings may therefore provoke new thought on established concepts of CSF circulation and the function of the brain's own absorptive organs (ependyma, arachnoid villi, choroid plexuses or ependymal organs)

ZUSAMMENFASSUNG

Molekulare und partikuläre Lösungen erreichen nach intrathekaler Applikation die zervikalen Lymphknoten *nicht nur schneller sondern auch in weitaus höhe*rer Konzentration als über den Blutweg. Auch scheint es, daß die Rückresorption über die Plexus chorioidei und die Absorption in den Arachnoidalzotten nicht so schnell verläuft wie der direkte Abfluß des Liquors ins Lymphsystem des Kopf-Hals-Gebietes. Unsere Untersuchungen lassen nicht nur eine weit offene Verbindung zwischen Liquor und Perilymphe einerseits und Liquor und Lymphsystem andererseits *erkennen, sondern führen zu der Annahme, daß die* tiefen Halslymphknoten eine wichtige Stelle der Liquor-Blut-Schranke darstellen müssen.

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Acta Otolaryng 76 162–172, 1973

MORPHOLOGY OF THE VESTIBULAR NERVE

I *Anatomical Studies of the Vestibular Nerve in Man*

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(Received February 22, 1973)

Abstract The intratemporal portion of the vestibular nerve in man has been studied with regard to its *gross and topographical anatomy The study includes* a light microscope investigation of the vestibular ganglion and the preganglionic parts of the vestibular nerve. The form and structure of the vestibular ganglion are described as well as the interrelation of ganglion cells and nerve branches. It was found that there is one ganglion composed of two major cell groups connected by a narrow isthmus. It was also found that in nearly all of the nerve specimens examined the posterior ampulla was innervated by two nerve branches. The present investigation is part of a major study of the vestibular nerve It provides the basis for further detailed studies of the vestibular nerve branches *regarding the number of nerve fibers* and the caliber spectra in the different branches.

The anatomy of the vestibular nerve has been subject of several investigations. Many ies of mammals including man have been tudied and found to have great structural similarities (Retzius, 1884 Voit 1907 Lorente de Nó 1926 Anson et al. 1967 Gacek, 1969 Sando et al. 1972, and others). The vestibular nerve is part of the VIII cranial nerve which according to international nomenclature is named the vestibulocochlear nerve or n. vestibulocochlearis. It is mainly composed of afferent fibers from the vestibular sensory regions to the vestibular nuclei. It also contains efferent fibers to the vestibular regions (Petroff 1955 Rasmussen & Gacek 1958 Gacek 1960, 1966) and to the cochlea (Rasmussen 1942, 1946) The vestibulocochlear nerve and the facial nerve course together from the brain stem through the internal acoustic porus into the internal auditory meatus. The afferent vestibular neurones are bipolar the ganglion cells are located in the vestibular ganglion at the bottom of the meatus. Two main portions of the ganglion can be distinguished pars superior and pars inferior and they are connected by the narrow isthmus ganglionaris (Alexander 1899). Wersäll (1956) found ganglion cells not only in the ganglion in the guinea pig but also scattered in the isthmus and in the nerve branches. According to Ballantyne & Engström (1969), however the ganglion cells in mammals form only one ganglion although a few ganglion cells are occasionally seen at some distance from the ganglion. The vestibular ganglion cells are generally larger and show greater variations of their diameters than the cochlear ganglion cells (Alexander 1899 Kellerhals et al., 1967 Ballantyne & Engström, 1969).

According to several authors, there is a tonotopic organization of the vestibular ganglion cells (i.e the functionally different areas of the sensory regions have determined projections in the vestibular ganglion as well as in the brain stem nuclei). Lorente de Nó (1926) recognized five regions in the vestibular ganglion of rat as distinguished by the size of their ganglion cells. Each region supplied a specific part of the sensory regions. Tonotopic organization of the vestibular ganglion has also been reported in monkey (Stein & Carpenter

This work has been sponsored in part by NASA Grants No. NGR 52-028-003 and NGR 52-028-004 and the University of Uppsala.

1967) and cat (Gacek, 1969). These authors found 2/3 of the ganglion cells in the superior and 1/3 in the inferior portion.

At the level of the ganglion and further peripherally the vestibular nerve is divided into a superior and an inferior division. The superior division innervates the sensory epithelia of the lateral and anterior ampullae, the utricle, and a small portion of the macula sacculi. The inferior division supplies the main portion of the macula sacculi and the posterior ampulla. The innervation of a small part of the macula sacculi from the superior division of the vestibular nerve was first reported by Retzius (1881) in teleosts and by Voit (1907) in mammals. The small nerve is usually called Voit's nerve. In mammals, including man, there is a connection between the inferior division of the vestibular nerve and the cochlear nerve: the vestibulo-cochlear anastomosis (Oort, 1918). It contains efferent fibers to the cochlea (Rasmussen 1942, 1946) as well as some afferent fibers (Rasmussen, 1953). A connection between the lateral ampullary nerve and the utricular nerve was described by Shute (1951). It was thought to contribute to the innervation of the macula utriculi. An anastomosis between the facial nerve and the VIII nerve was reported by Lorente de Nó (1926). These fibers were shown to leave the facial nerve at the level of the proximal part of the geniculate ganglion (Shute, 1951). There is also an anastomosis between the vestibular and facial nerves. It belongs to the intermedius nerve (Gacek & Rasmussen 1961) which is adherent to the VIII nerve for a variable distance (Rhoton et al. 1968).

Surgical interventions on the vestibular nerve and the inner ear such as the removal of intrameatal acoustic neuromas, vestibular nerve section in Menière's disease and shunt operations are no rareties today. They require a thorough knowledge of the anatomy of the temporal bone. The aim of the present investigation was to study and illustrate the gross and topographical anatomy of the intratemporal part of the vestibular nerve in man. The vestibular ganglion and the preganglionic parts of the vestibular nerve have been studied in detail as a basis for further numerical studies of the vestibular nerve.

MATERIAL AND METHOD

The observations of the general form and topographical relationships of the vestibulocochlear nerve have been made on 40 temporal bone specimens. The left temporal bone with the VII and VIII nerves was removed at autopsy and fixed in 10% formaldehyde. Soft tissues were excised and the bone decalcified in 8% HNO_3 for 10–14 days. After neutralization in 5% Na_2SO_4 for 24 hours the specimen was rinsed in water and transferred to and stored in 70% alcohol.

The dissections were performed under a Wild M 5 dissection microscope with the specimens in 70% alcohol. The decalcified labyrinthine capsule as well as the bone surrounding the internal auditory meatus were easily removed with the aid of knives and small forceps. The vestibulocochlear and facial nerves as well as the membranous labyrinth were thus exposed and studied. Photographs of the preparations were taken on Kodak Tri-X film with a Hasselblad 500 C camera using an 120 mm lens and extension tubes of varying lengths.

The light microscope studies of the vestibular nerve fibers and ganglion were performed on 11 nerve specimens used in a numerical study. Methods of fixation, embedding, sectioning and staining are fully described in that study (Bergström 1973). One more nerve specimen

Table I. *Measurements of the internal auditory meatus*

Case	Hor diam (mm)	Vert diam (mm)	Length (mm)
Newborn	6	4	7
6 weeks	4.5	2.5	7
F 80 yrs	5	—	10
F 80 yrs	5	—	1
M 78 yrs	8	—	17
M 35 yrs	7	—	12
M 49 yrs	7	7	11

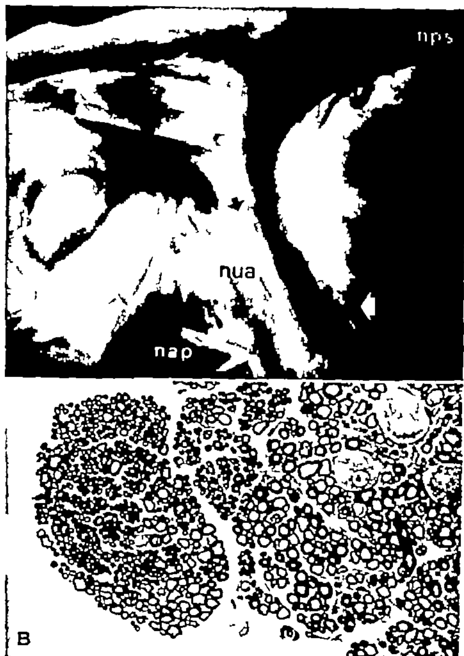


Fig 1 (A) Superior view of the labyrinth, the vestibulocochlear nerve and the facial nerve. (*aa*) anterior ampulla. (*nua*) utriculo-ampullary nerve (*nap*) posterior ampullary nerve note thin accessory branch on proximal side. (*VII*) facial nerve. Black arrow indicates vestibulo-facial anastomosis. (*nps*) superficial petrosal nerves leaving the geniculate ganglion. Cochlear nerve at white arrow × 14 (B) Section through the superior portion of the vestibular ganglion where the fibers of the vestibulo-facial anastomosis can be seen to the left. Most of these fibers have much smaller diameters than the vestibular nerve fibers. 260.

was treated in the same manner and horizontally sectioned through the vestibular ganglion Reconstructions of the vestibular ganglion were made with the aid of a microscope image projector

OBSERVATIONS

Form and course of the vestibular nerve

In this presentation the head is assumed to be bent forward 30 (i.e the lateral semicircular canal will be in the horizontal plane). The



Fig. 2. Relations between superior ampullae utriculo-ampullary nerve, facial nerve, and oval window (*aa*) anterior ampulla (*al*) lateral ampulla. Arrows indicate membranous labyrinth within the perilymphatic space. (*sas*) superior ampullary nerves. (*un*) utricular nerve. (*VII*) facial nerve. (*st*) stapes. 12.

vestibulocochlear nerve leaves the brain stem and the cranial cavity together with the facial nerve through the internal acoustic porus. Surrounded by a common dural sheath they proceed in the internal auditory meatus to reach the different sensory regions of the labyrinth.

The horizontal diameter of the porus and the length of the meatus from porus to the vertical crest dividing the facial nerve from the superior vestibular nerve division were measured in five non-decalcified temporal bones from adults and in one newborn and one 6-week-old child. In the adults the horizontal diameters varied between 5 and 8 mm and the length of the meatus between 10 and 17 mm. In the newborn child the horizontal diameter was 6 mm, the vertical diameter 4 mm ,and the length of the meatus 7 mm. Corresponding values for the 6-week-old child were 4.5 mm, 2.5 mm and 7 mm (Table I). These observa tions agree with those of Papangelou and others (Papangelou 1972).

The facial nerve is positioned antero-su periorly in the meatus with the cochlear nerve inferior to it while the vestibular nerve runs posterior to these two nerves (Fig. 1 A). The vestibular nerve divides into a superior and an inferior division. The superior courses together with the facial nerve over the sharp horizontal ridge (the transverse crest) which divides the fundus region in two halves. The inferior vestibular nerve division and the cochlear nerve pass under this crest.

The facial nerve lies very close to the superior vestibular nerve division in the meatus. Peripherally the two nerves leave the meatus through bony canals separated by a sharp vertical crest. Proximal to this crest a connection between the nerves can be seen

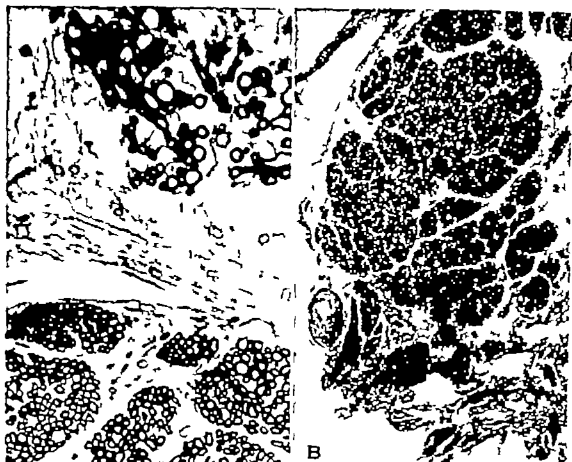


Fig 3 (A) Section through the anterior ampullary crista in a 22-year-old woman. The nerve fibers are arranged in bundles as they pass through the bone. e fibers in the upper part of the section have inctly greater diameters than those in the lower rt × 425 (B) Cross-section through superior division of the vestibular nerve peripheral to the ganglion. Anterior and lateral ampullary nerves (*aa*) are inseparable at this level. The utricular nerve (*u*) with its inferior bundles penetrating the labyrinthine capsule. The blood vessel at the anterior border is a constant finding. × 63

(the vestibulo-facial anastomosis) The fibers of the vestibulo-facial anastomosis are generally much thinner than those of the vestibular nerve although a few thick fibers occur Fig. 1 B shows the vestibulo-facial anastomosis in a 53-year-old man. It contains about 700 fibers. The facial nerve proceeds in its bony canal in an anterior and lateral direction to the geniculate ganglion After emitting the major and minor superficial petrosal nerves the facial nerve bends sharply in a posterior direction and passes lateral and immediately inferior to the lateral ampulla to proceed between the lateral semicircular canal and the oval window (Fig 2). The superior division of the vestibular nerve runs in a bony canal on the labyrinthine capsule. The utricular nerve goes off in a postero-inferior direction while the fibers of the two superior ampullary nerves form one single nerve branch during the greater part of their course. They do not divide into two separate branches until just proximal to the ampullae. Immediately distal to the vestibular ganglion, which reaches far laterally in the superior division, the thick fibers are rather evenly dispersed over the cross section area. At more peripheral levels the thick fibers tend to gather towards the center of the area and when the two branches have divided they are seen as fairly distinct bundles in the center of



Fig. 4 (A) Posterior view of the eighth cranial nerve in man. (nua) utriculo-ampullary nerve, (ns) saccular nerve (nap) posterior ampullary nerve, (nc) cochlear nerve entering the cochlea. 16. Inset shows location of vestibular ganglion. (B) Posterior ampullary nerve with accessory branch seen from below. In this case the accessory branch is seen distal to the main branch. 20.

the branches. The thinner fibers are then found predominantly in the periphery of the cross section area. In a section through the anterior crista (Fig. 3 A) the fibers are arranged in distinct bundles with the thickest (14–15 microns) located together.

The utricular nerve lies inferior to the superior ampullary nerves. It has a rather short course as an independent nerve branch during its passage from the ganglion to the foraminous nerve entry in the labyrinth capsule. The fibers are more densely packed and most of them are thinner than in the ampullary nerves although a certain number of



Fig 5 Membranous labyrinth and eighth nerve seen from below (*ap*) posterior ampulla. (*nap*) posterior ampullary nerve with accessory branch. (*ns*) saccular nerve. (*nc*) cochlear nerve (*nps*) superficial petrosal nerves. (*s*) head of stapes. Round window is seen beneath stapes. × 10.

thick fibers occur all over a cross-section area the nerve (Fig 3 B).

In a small canal in the superior surface of transverse crest a thin nerve branch can be followed to the macula sacculi. This is known as Voit's nerve and sometimes it is dehiscent in the proximal part of its course on the crest.

The main portion of the inferior vestibular nerve division continues in the direction of the nerve stem as the saccular nerve. It passes under the transverse crest down to the bottom of the internal meatus where it enters the labyrinth to innervate the macula sacculi. In this branch as in the utricular nerve there is a predominance of thin fibers and only a few thick fibers are seen.

In one of the microscopically investigated cases a nerve branch much thicker than Voit's nerve was observed to leave the saccular nerve distal to the vestibular ganglion and course over the transverse crest to reach the macula sacculi that way. There was also in this case a thinner branch from the superior surface of the saccular nerve anterior to the thicker branch. This thinner nerve branch passed under the crest and could be followed to the foramina of the macula sacculi.

The second branch of the inferior vestibular nerve division (the posterior ampullary nerve) emerges from the main stem inside the meatus and leaves the meatus through a bony canal in a postero-lateral direction to reach the labyrinthine capsule which it follows to the posterior ampulla. The nerve branch is composed of 10–20 rather loosely arranged bundles in which the nerve fibers are closely packed. There are great variations in fiber thickness but as in the anterior and lateral ampullary nerves, the thick fibers dominate.



Fig. 6. (A) Reconstruction of the vestibular ganglion as seen from the posterior side. Schematic drawing of the vestibular nerve with superior (*sup*) and inferior (*inf*) divisions. (B) Location of the vestibular ganglion in the vestibular nerve. (*nal*) lateral ampullary nerve. (*naa*) anterior ampullary nerve. (*nu*) utricular nerve. (*ns*) saccular nerve. (*nap*) posterior ampullary nerve. (C) Horizontal section through the inferior portion of the vestibular ganglion. Proximal end to the right. (*ns*) saccular nerve. (*nap*) posterior ampullary nerve. ×25

In practically all cases examined the posterior ampullary nerve was found to have a thin accessory nerve. This branch left the main stem of the vestibular nerve 1–2 mm proximal and slightly superior to the main branch to the posterior ampulla. In one case is was seen to originate from the posterior ampullary nerve inside the meatus and in another case it emerged from the distal surface of the posterior ampullary nerve and went separately to the ampulla (Fig. 4 B). In all cases except the last mentioned one the thin accessory branch left the internal auditory meatus through a separate bony canal immediately proximal and slightly superior to the foramen singulare. After 2–3 mm it usually united with the main posterior ampullary nerve and they reached the ampulla together but in a few cases the thin branch ran a separate course all the way to the ampulla.

Form and structure of the vestibular ganglion

The vestibular ganglion is located near the bottom of the internal auditory meatus. It is divided into one superior and one inferior portion (pars superior and pars inferior) held together by the narrow isthmus ganglionaris (Fig. 6 A).

The main direction of the ganglion is an oblique line from the supero-antero-lateral side to the infero-postero-medial side. Pars superior is the greater of the two portions. It contains the ganglion cells of the lateral and anterior ampullary nerves and those of the utricular nerve. In the present study it was not possible to determine the origin of Voit's nerve. Pars inferior holds the ganglion cells of the saccular and posterior ampullary nerves.

The ganglion cells are closely packed and they form a rather distinct ganglion although a few scattered cells are seen at some distance from this cell mass. No bulging of the outer contour of the vestibular nerve in the manner so often presented in drawings of the vestibular ganglion was seen. There was, however, a small bulging of the anterior margin of pars superior, isthmus and the superior part of pars inferior.

The superior ganglion cells reach farthest peripherally in the supero-lateral margin of the lateral ampullary nerve and in the supero-medial margin of the anterior ampullary nerve. The most peripheral ganglion cells are found approximately 3 mm proximal to the anterior ampulla. More proximally the ganglion cells occupy the whole cross-section area of the superior vestibular nerve division. Still more proximally the ganglion narrows in a postero-inferior direction. Thus pars superior becomes

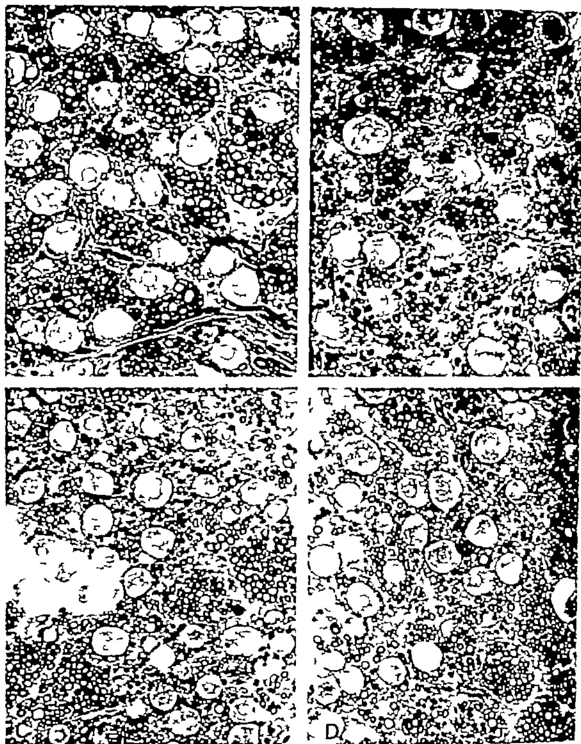


Fig. 7 Cross sections through the vestibular ganglion. (A) Central part of the superior portion in a 35-year old man. (B) Upper part of the superior portion in a 22 year-old woman. (C) Anterior part of the inferior portion in the same case. (D) Posterior part of the inferior portion in the same case. × 200.

more or less "Y" -shaped when sectioned in the horizontal plane. When sectioned longitudinally in the sagittal plane it is seen as a narrowing band in the proximal and inferior direction down to the isthmus region. In a horizontal (10 microns thick) section pars superior has a

maximum length of about 3 mm and the "legs" of the "Y" are 0.8–1 mm wide.

Isthmus ganglionaris has a length of 0.4–0.5 mm and a width of about 1 mm. It is in contact with the central region of pars inferior which has a more elongated and slender form than pars superior. Its total length is 4–4.5 mm. The saccular ganglion cells are located anterior and peripheral to those of the posterior ampullary nerve. No ganglion cells were found in the posterior ampullary nerve outside the main vestibular nerve stem.

The vestibular ganglion cells have a rich supply of small blood vessels which can be seen branching around the ganglion cells. The ganglion cells are almost round or ovoid in form with the nucleus more or less in the center. The diameters of the ganglion cells vary between 15 and 50 microns and the majority seem to be 30–40 microns in diameter. Regional differences in cell size were not sufficiently evident to allow a subdivision of the ganglion based on the size of the ganglion cells.

DISCUSSION

In the superior division of the vestibular nerve the branches to the anterior and lateral ampullae are almost inseparable during the greater part of their course. They divide into separate branches very close to the ampullae. Thick and thin fibers occur all over the cross section area close to the ganglion but at more distal levels the thick fibers are more centrally located. Inside the crista the thickest fibers run in separate bundles. They seem to be Cajal's (1909) "fibres colossales" and Lorente de Nó's (1926) "fibres grosses". According to these authors the colossal fibers went to the hair cells on the top of the crista where they innervated one single or small groups of two or three sensory cells.

The relatively thick nerve branch that was seen to emerge from the saccular nerve in one case and pass over the transverse crest to the macula sacculi is probably the same branch that Lindeman (1969) described in a number of species including man. There was also in this case a thinner branch that left the saccular nerve at the same level. This smaller branch coursed under the transverse crest to the macula sacculi and might be the cochleo-saccular nerve to the posterior part of the macula sacculi described by Hardy (1934) and shown by Shute (1951) to come from the vestibular ganglion.

In nearly all of the investigated specimens the posterior ampullary nerve was found to be followed by a thin accessory branch. In most cases it left the vestibular nerve stem proximal and slightly superior to the main branch to the posterior ampulla through a separate foramen in the wall of the internal meatus. Gacek (1961) observed a double innervation of the posterior ampulla in 4 human temporal bones. Montandon et al. (1970) reported two cases of crista neglecta in man where they observed an accessory branch to the posterior ampulla. This branch was said to leave the nerve stem distal to the main posterior ampullary nerve. They studied more than 600 temporal bones and in all of them two posterior ampullary nerves were found. In the present investigation the course of the nerve fibers was not studied inside the cristae.

The vestibular ganglion cells form one, irregularly contoured, ganglion consisting of two main portions, pars superior and pars inferior. The proximal part of the superior portion of the ganglion was connected with the central region of the inferior portion by a narrow isthmus. The vestibular ganglion cells varied in size between 15 and 50 microns. It was not possible to make a reliable subdivision of the vestibular ganglion into different regions based on the cell size as was done by Lorente de Nó (1926).

ZUSAMMENFASSUNG

Der intratemporale Teil des Vestibularnervs bei Menschen wurde hinsichtlich seiner makroskopischen und topographischen Anatomie untersucht. Die Untersuchung umfasst auch mikroskopische Studien des

Vestibularganglions und des präganglionaren Teils des Vestibularnervs. Die Form und die Struktur des Vestibularganglions sowie die Beziehungen zwischen Ganglienzellen und Nervenästen werden beschrieben. Es stellte sich heraus, dass es sich um ein Ganglion bestehend aus zwei grossen Zellgruppen handelt die durch einen schmalen Isthmus verbunden sind. In fast allen untersuchten Nervenpräparaten war Ampulla posterior von zwei Nervenästen innerviert. Die vorliegende Untersuchung stellt einen Teil einer grösseren Studie über die Vestibularnerven dar. Sie bildet die Basis für weitere detaillierte Studien der Vestibularnervenäste deren Faseranzahl und der Kaliberverhältnisse der verschiedenen Äste.

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Acta Otolaryng 76 173–179 1973

MORPHOLOGY OF THE VESTIBULAR NERVE

II *The Number of Myelinated Vestibular Nerve Fibers in Man at Various Ages*

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(Received February 22, 1973)

Abstract This paper contains a study of the vestibular nerve and its branches in man at various ages. It has been done with the purpose of estimating the "normal" number of nerve fibers and to investigate whether any reduction occurs with increasing age. The vestibular nerves from 11 individuals of varying ages ranging from birth to 85 years were examined. There was no known history of vestibular disorders such as Menière's disease or acoustic neuroma, or of treatment with ototoxic antibiotics or irradiation therapy to the head. Four persons in a younger group up to 35 years, had between 16 040 and 20 212 (average of 18 346) myelinated vestibular nerve fibers. The number of nerve fibers in 5 old individuals, 75–85 years, ranged between 9 274 and 15 980 with an average value of 11 506. The study has thus shown a considerable reduction of the number of vestibular nerve fibers with increasing age. The reduction in number of nerve fibers in the old age group averages 37% and is statistically significant at better than the 1% level.

Numerical studies of the human vestibular system have been done by Rasmussen (1940), Naufal & Schuknecht (1972) and Rosenhall (1972 *a*, 1972 *b* 1973). Rasmussen (1940) examined 37 vestibular nerves at an intracranial level. In the age group 2 to 26 years, fiber counts ranged between 15 300 and 24 000 with an average number of 18 900 In the age group 44 to 60 years he found from 14 200 to 22 900 fibers and the average value was 18 000

Naufal & Schuknecht (1972) counted the number of vestibular ganglion cells in 15 vestibular nerves from 9 individuals of different ages with no history of ear disease or hearing loss. They found an average number of 18 439 vestibular ganglion cells (range 14 920–22 390) with no signs of age related degeneration. In a further case 86 years old with diabetes mellitus from the age of 40 they found 12 430 ganglion cells in the right and 10 910 in the left vestibular nerve. In all but one case the majority of the ganglion cells were reported to belong to the inferior division of the nerve. In Stein & Carpenter's (1967) study on monkey 2/3 of the nerve fibers belonged to the superior vestibular ganglion cells. In a numerical analysis of the vestibular nerve and its rami by Gacek & Rasmussen (1961) the ratio of nerve fibers in the superior and inferior divisions was approximately 3 2 in the guinea pig cat, and monkey

The vestibular sensory cells have been analysed by Rosenhall (1972 *a* 1972 *b* 1973). In a group of individuals, none of whom was older than 40 years, each of the three cristae had an average number of 7 600 hair cells (range 6 700–8 300) The macula utriculi carried 33 100 (29 500–39 200) hair cells and the macula sacculi 18 800 (16 000–21 300) In his old age group quite clear degenerative changes were found. The reduction in number of sensory cells amounted to about 40% on the cristae and approximately 20% on the maculae. There were great individual variations in individual losses. Apart from Rosen-

This work has been sponsored in part by NASA Grants No. NGR 52-028-003 and NGR 52-028-004 and the University of Uppsala.

hall's reports, the existence of old age related degenerative changes in the vestibular system has been denied by a number of authors. v *Fleandt & Saxén (1937)* Jörgensen (1964) Hansen & Reske-Nielsen (1964), Reske-Nielsen & Hansen (1964) have all studied the degenerative processes of the cochlea in aging. In that connection they also looked at the vestibular system without finding any changes that could be related to old age.

Schuknecht (1964) presented four cases with extensive degeneration in the cochlea attributed to old age In one of the cases, he reported degeneration of the macula sacculi while the rest of the vestibular system was said to be normal. In two of the cases the whole vestibular system was reported to be normal and in the fourth case there was no information *about the vestibular part. Schuknecht et al.* (1965) found what they called cochleo-saccular degeneration in an old cat, an old dog, and an old man. All these had extensive age related degeneration in the cochlea and losses of about 50% of the hair cells of the macula sacculi The other vestibular sensory regions appeared to be normal. They drew the conclusion that the phylogenetically younger pars inferior of the labyrinth is more vulnerable to . than the phylogenetically older pars sup- Johnsson (1971) and Johnsson and Hawons (1972 *a*) found age-related degenerations in the macula sacculi but only minor changes in the macula utriculi. However none of those reports was based on numerical studies and it therefore seemed worthwhile to make a numerical analysis of the vestibular nerve *The purpose was to estimate what might be termed the "normal" amount of myelinated fibers in the vestibular nerve and its branches and to ascertain any reduction in number with increasing age* A preliminary report of the results obtained has been published (Bergström 1972).

MATERIAL AND METHOD

The left vestibular nerve from 11 individuals aged from birth to 85 years was obtained at autopsy In no case was there a known history of vestibular disorders such as Menière's disease or acoustic neuroma, treatment with ototoxic antibiotics or irradiation therapy to the head. One case with leukemia (22 years) had been treated with the cytotoxic agent cyclophosphamid (Sendoxan®) a few months prior to death. But since she had one of the highest numbers of nerve fibers counted she has been included in the series.

At autopsy formaldehyde-eosin was injected through the carotid artery (as a part of the embalming procedure) and the left temporal bone was then removed care being taken not to damage the intra temporal parts of the VII and VIII nerves. Soft tissues were excised and the bony roof of the internal auditory meatus was drilled away in order to expose *the nerves. The middle ear was opened, the* ossicles removed and the oval and round windows were opened The specimen was placed in 2.5% glutaraldehyde for about 24 hours. The specimen was moved to saline and the vestibulocochlear nerve with its branches was very carefully dissected and removed from the temporal bone The nerve specimen was then fixed in cold, Veronal-buffered 1.5% OsO_4 for 2 hours at +4 C. After rinsing in water dehydration took place in increasing concentrations of alcohol and propylene-oxide. The nerves were then embedded in Epon 812. If for some reason the whole procedure could not be carried out without interruption, intervals were made at either the stage of glutaraldehyde, 70% alcohol or propylene-oxide-Epon.

Altogether 31 temporal bones were collected but in some of these the nerves had been torn peripherally when the brain was removed from the cranial cavity In some of the first cases the nerves could not be dissected free in a satisfactory manner In one case of acute *intracranial hemorrhage* the histologic sections were obscured by an extravasation of blood into the nerve. Another temporal bone had large blood clots in the internal auditory *meatus and was discarded.*

The sectioning was done with a glass-knife

with a LKB Ultrotome. Cross-sections of the nerve branches, 3 μ in thickness, at various levels peripheral to the vestibular ganglion were stained with paraphenylene-diamine. After rinsing and dehydration they were mounted in Canada balsam. A Wild M 20 microscope was used for the numerical studies which were made at 500 × magnification with the help of an ocular micrometer disc divided into 100 squares. The nerve fibers touching the left and upper margins were not counted while those touching the right and lower margins were included. It was possible to move the specimen under the microscope so precisely that every fiber within the whole nerve section could be counted.

Since the vestibular nerve fibers occur in bundles that are irregularly separated by connective tissue and blood vessels, no sampling procedures could be used and it was necessary to count all fibers.

In 5 cases, control analyses were made a few months after the initial counts without knowledge of the previous results the differences were less than 1 %

The anterior and lateral ampullary nerves run together for the greater part of their course and could not be reliably separated until just proximal to the ampullae. Since it was not always possible to get representative cross-sections of these branches at that level, the two superior ampullary nerves have been counted as a unit on cross-sections immediately peripheral to the ganglion. The utricular nerve could be distinguished from the two superior ampullary nerves in all but 3 cases. In these 3 cases the *superior division of the vestibular* nerve is accounted for as a unit. The saccular nerve offered no greater problems regarding demarcation. The fibers of the olivo-cochlear bundle leave this nerve branch at the level of the vestibular ganglion and thus did not interfere.

The posterior ampullary nerve was dissected from its bony canal in 7 cases and analysed at the level where the thin accessory branch (Bergström. 1973) connects with it. In the other 4 cases the intrameatal part of this nerve branch was taken out together with the whole vestibular nerve. In 3 cases the fibers of the accessory branch were counted separately (131 284 336).

The material has been grouped according to age. The first group (young group) consists of 4 individuals, 1 day 6 weeks, 22 years, and 35 years old. In the second group (middle age group) are the 2 middle-aged cases, 49 and 53 years old, and the third group (old age group) contains 5 cases, 75–85 years old.

RESULT

Young group

An can be seen in Table I the total number of vestibular nerve fibers in this group averages 18 346. When the different nerve branches are analysed it is found that the average value for the two superior ampullary nerves, which for reasons stated above have been counted as a unit, is 5 899 fibers. The utricular nerve contains 5 952 fibers, the saccular nerve 4 046 and the posterior ampullary nerve 2 449 fibers. Two-thirds of the fibers belong to the superior division and 1/3 to the inferior There are pronounced individual variations regarding the number of nerve fibers especially in the superior division. Although all three cristae are reported to have the same number of sensory cells (Rosenhall, 1972 *b*) the ratio between the two superior ampullary nerves and the posterior one is not found to be 2 1 in this material. In 1 case (6 weeks) the given value for the posterior ampullary nerve, 2 124 is *probably too low as a small number of fibers* in the periphery seem to have been torn away at preparation. In 1 individual (22 years) one relatively thick nerve branch containing 953 fibers and one thinner with 289 fibers, left the saccular nerve immediately distal to the ganglion. The thicker branch passed over the transverse crest and reached the macula sacculi, while the thinner branch ran under the crest to the macula sacculi. They have been discussed in a previous study (Bergström, 1973)

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Table III *Numerical data on the vestibular nerve and its rami in the old age group*

	Superior division			Inferior division			
Age	Nn amp ant et lat	N utr	Total	N sacc	N amp post	Total	Total vest. nerve
75	—	—	11 122	2 779	2 079	4 858	15 980
78	3 001	2 599	5 600	1 973	1 701	3 674	9 274
80	2 618	3 360	5 978	[illegible] 784	1 443	4 227	10 205
80	—	—	7 034	1 907	1 133	3 040	10 074
					1 161		
85	4 308	2 695	7 003	3 500	+ 336	4 997	1 000
Mean	(3 309)	(2 885)	7 347	589	1 570	4 159	11 506

apy to the head. One young person had been treated with a cytotoxic agent but showed one of the highest numbers of nerve fibers in the material.

The material was arranged in three age groups with the purpose of establishing what might be termed the "normal" number of vestibular nerve fibers and to evaluate the magnitude of the age related reduction of nerve fibers. The young or "normal" group included subjects with ages from birth to 35 years. This is in accord with Rosenhall s observations of the vestibular sensory cells where he found no sizeable reduction of hair cell populations before the age of 40 In the present study however there seems to be a certain reduction of nerve fibers in the 35-year-old man. Since individual variations regarding the number of nerve fibers are likely to occur he has been included in the "normal" group. If this case had been placed outside the young group an even earlier and greater reduction in nerve fiber count than is reported here would be present.

The normal" values found in this study (18 346) agree very well with those of earlier reports; 18 900 (Rasmussen, 1940) 18 439 (Naufal & Schuknecht, 1972).

The two middle aged cases showed a slight reduction of their numbers of vestibular nerve fibers while in the old age group" (75–85 years) a decrease in fiber counts of about 37 was found as compared with the "young group"

The reports of an increasing loss of hair cells in the vestibular sensory regions with increasing age when combined with the observed numerical reduction of vestibular nerve fibers in old age found in the present investigation indicate an important correlation of morphological findings within the inner ear

It thus seems that a general reduction of the number of vestibular sensory cells and their nerve fibers takes place with increasing age and usually begins around the age of 40. The vestibular parts of the inner ear and VIII nerve undergo pronounced degeneration with increasing age in the same manner as has long been known to occur in the acoustic parts. From this point of view the recently reported observations on age related changes of the

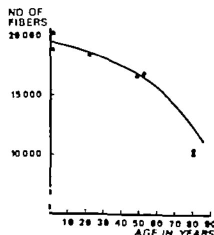


Fig. 1 Correlation between age and number of vestibular nerve fibers in the investigated individuals.

vestibular function (van der Laan, 1972) are very interesting. Different vestibular tests were performed on healthy subjects of various ages. With all these tests, age-related changes were found. The caloric test revealed an increased response up to the age of 40 followed by a decline with increasing age. Possible explanations of the observed age affects were thought to be habituation vascular influences and vestibular nerve degeneration. It seems likely that elderly people can suffer from vestibular disorders due to degeneration of sensory cells and nerve fibers. The fact that vertigo and balance problems are common in old age is probably not only caused by these peripheral lesions but also by injuries to the central vestibular areas. Vascular changes in the vessels of the internal auditory meatus and the inner ear with aging have recently been reported (Fisch et al., 1972 Johnsson & Hawkins, 1972 *b*)

From a clinical physiological point of view degenerative changes have been reported to occur in old age. Arslan (1957) and Minnigerode et al. (1967) have observed decreased caloric responses with increasing age. Bruner & Norris (1971) found an increasing caloric responsitivity up to 60 years of age and a progressive decrease in responsitivity in patients er that 60 They suggested that the observed e effects were caused by vascular influences and loss of central inhibition. In Arslan s study a few cases with increased vestibular responsitivity occurred, which he thought might be caused by a conditioned decrease of the central inhibition exerted by different supranuclear structures due to arteriosclerotic changes. Increased rotatory threshold in old age is reported by Haas (1964)

The present study has only been concerned with the number of myelinated fibers in the vestibular nerve. It is probable that the numerical reduction of vestibular nerve fibers is accompanied by alterations of the remaining ganglion cells and nerve fibers. With the present results in mind it should be of great interest to investigate any relationship between sensory cell damage and nerve fiber alteration.

Preliminary observations in this study indicate that the thick fibers from the ampullary cristae suffer the greatest losses in old age but further observations will be needed. From this investigation it is also evident that some elderly persons can retain an almost normal population of fibers in the vestibular nerve while other individuals may show extensive reductions of their nerve fiber counts. These findings constitute a very interesting basis for further clinical studies of vestibular reactivity and central compensating mechanisms.

ZUSAMMENFASSUNG

Die vorliegende Arbeit enthält eine Studie über Vestibularnerven und deren Äste bei Menschen verschiedenen Alters. Das Ziel war es, die „normale" Anzahl der Nervenfasern zu bestimmen und zu untersuchen, inwieweit eine Reduktion der Faseranzahl bei steigendem Alter auftritt. Die Vestibularnerven von 11 Individuen verschiedenen Alters, von Neugeborenen bis zu 85jährigen sind untersucht worden. In keinem Fall lagen irgendwelche Angaben über vestibulare Krankheiten (wie Menière oder Akustikustumoren oder über Behandlung mit ototoxischen Antibiotika oder Strahlenbehandlung des Kopfes vor 4 Personen in einer „jungen Gruppe" (bis zu 35 Jahren) hatten zwischen 16 040 und 20 212 (durchschnittlich 18 346) myelinisierte Vestibularnervenfasern. Die Anzahl Nervenfasern bei 5 alten Personen (75–85 Jahre) variierte zwischen 9 274 und 15 980, mit einem Durchschnitt von 11 506. Die Untersuchungen haben folglich eine erhebliche Reduktion der Anzahl Vestibularnervenfasern mit steigendem Alter gezeigt. Die Vestibularnervenfasern bei der Gruppe mit alten Personen nahmen durchschnittlich bis zu 37% ab. Die Abnahme liegt statistisch auf einem Niveau on 1%

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Acta Otolaryng 76 180–182, 1973

FALSE NEGATIVE RESPONSE FROM CALORIC STIMULATION

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(Received November 30 1972)

Abstract Greisen (1972) has recently drawn attention to "pseudocaloric nystagmus" The present authors disagree with his interpretation of the evidence he puts forward, but offer an alternative explanation of his evidence and a method for detecting the phenomenon when it occurs. Their arguments also indicate the undesirability of shortening the bithermal caloric test to one temperature only and the advantages of doing the hot tests before the cold ones.

In a recent paper (Greisen 1972) attention was drawn to the occasional occurrence of nystagmus when a functionless vestibular labyrinth is stimulated by a caloric test irrigation (pseudocaloric nystagmus). The partial compensation of destruction nystagmus that ormally occurs after a few weeks may be uced by various sensorial stimuli, such as loric irrigation, giving rise to nystagmus hich is not a response to ampullary stimulation. Rubin & Vandemoer (1971) noted that the latency of such nystagmus was shorter than that of true caloric nystagmus, and that the nystagmus is in the same direction as any spontaneous nystagmus (SN) that may be present, but not necessarily in the same direction as that to be expected from the caloric stimulus.

Similarly a false negative effect may be obtained from caloric stimulation of a functioning labyrinth. Apart from inadequacy of irrigation (a not uncommon happening even in experienced hands, but which can be detected by looking for the "tympanic flush sign Coles 1972) it may also arise when an induced nystagmus is approximately equal in velocity but opposite in direction to a pre existing SN (or directional preponderance).

If caloric testing is performed with the aid of electronystagmographic recording without visual fixation and with mental arithmetic (reinforcement) any SN occurring under the caloric test conditions may in most cases be detected, and measured with greater sensitivity than by direct observation. It can also be allowed for when measuring the response to caloric irrigation, as illustrated in Fig. 1 and may thereby help to reduce the apparent incidence of one absent response and the difficulty of interpreting such an absent response.

Unfortunately the example of pseudocaloric nystagmus in Greisen's article (Fig. 2) does not appear to have been tested with electronystagmography Had this been done a SN might have shown up in the absence of the inhibiting effect of visual fixation Fig 2 could well be an example of a false negative response resulting from a combination of an R canal paresis (CP) and an undetected spontaneous nystagmus to the L or a directional preponderance (DP) to the L.

He reasons that the 30 C R response is pseudocaloric because there had been no response to stimulating that ear at 44 C in the usual supine position nor at 4 C in the prone position. We disagree If nystagmus following the 30 C irrigation of the R ear was pseudocaloric (i.e. there was no ampullary response

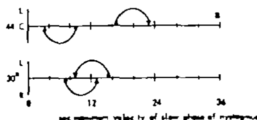


Fig. 1 Calorigrams showing modifications of canal pareses by two patterns of spontaneous nystagmus (or directional preponderance). (*a*) Interaction of a CP with a SN towards the opposite side (the more usual pattern). (*b*) Interaction of a CP with a SN towards the same side (the less usual pattern). Degree of nystagmus not recordable (by ENG without visual fixation, with reinforcement). ⌐ Nystagmus expected from CP alone. ▼ Nystagmus resulting from interaction of CP with SN.

whatsoever) why did not the 44 C irrigation of that ear produce a similar pseudocaloric response? Perhaps the hot caloric was regarded as less potent as a non-vestibular stimulus than the cold one. Yet why was there no response from a 4 C stimulus to the R ear either which certainly would be a more potent non-vestibular stimulus than that at 30 C? The answer must surely be that the 4 C stimulus was administered to the patient in the prone position. As with the hot test in the supine position, the stimulus would be an ampullo-petal one and produce a vestibular response which would be in opposition to the latent destruction nystagmus. On the contrary 30 C stimulus in the supine position would have produced an ampullo-fugal stimulus, the vestibular response to which would reinforce the hitherto latent destruction nystagmus to the L ear. It is contended that such a pattern of results is direct evidence *for* a real caloric response in the R ear and *against* a pseudocaloric one.

In spite of our objections to this evidence, as presented by Greisen, for the existence of pseudo-caloric nystagmus, we have ourselves observed phenomena that can only be explained on the basis of a pseudocaloric response. If there is any doubt as to the validity of a single response to a pair (44 C and 30 C) of caloric stimuli, the situation may be clarified by irrigation with water at 20 C in the supine position, which should increase the caloric (or pseudocaloric) response. The irrigation is then repeated in the prone position, which will reverse the direction of the nystagmus if it is caloric, but will not affect it if it is simply an arousal of a latent destruction nystagmus.

False-negative and apparent pseudocaloric responses occur more frequently when using direct observation than with ENG recording, which is one of the advantages to be gained from the latter procedure. This need to detect the presence of any pseudocaloric nystagmus and/or any DP and to allow for any SN or DP in interpreting caloric test results, reinforces the arguments for not shortening the test to one temperature only. Thus, in the

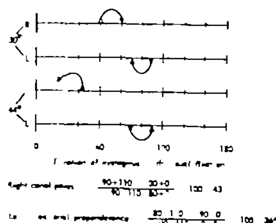


Fig. 2 Calorigram of Greisen's example of pseudocaloric nystagmus (modified from Greisen, 1972, who interpreted the 30°C right test as being pseudocaloric). Period in which nystagmus is not likely to be observable. ⌐ Nystagmus durations reported by Greisen. ▼ Present author's interpretation of the basic response of each labyrinth (having allowed for a presumed SN or DP to L).

sort of patterns of responses shown in Fig. 1 the R CP would certainly have been missed if hot tests only had been performed in case (*b*), or cold tests only in case (*a*)

Finally inspite of the desirability of doing caloric tests in each ear at both temperatures, it occasionally happens that the patient s cooperation is withdrawn before the tests are completed. It is therefore important to have performed the more useful ones first these we consider to be the hot ones, on the following grounds. (i) The pattern of inter action between CP and SN or DP shown in Fig. 1 (*a*) is considerably more common than that in Fig. 1 (*b*). (ii) The response to hot tests, when measured without visual fixation in terms of maximum velocity of slow phase of induced nystagmus, has been shown by Hinchcliffe (1967) to give the best indication of the severity of the pathological process. (iii) The normal response to hot stimuli is greater than to cold ones, when measured by maximum velocity of slow phase (as compared with duration of nystagmus, which leads to artefacts in tests performed without visual fixation) and hence may give more clear-cut results than from the cold tests. (iv) Being an ampullo-petal stimulus, in the con- ntional caloric test position, the hot test is, in, a more sensitive one and may give ordable responses in hyporeactive ears where there is absence of any apparent re sponse to cold tests. (v) If any negative re sponse occurs, its validity can be checked by looking for the "tympanic flush sign" which can usually be seen immediately after a hot irrigation but seldom after a cold one more over even if all four tests are performed, time may be wasted by not detecting at once any tendency towards inadequacy of irrigation, if the cold tests are performed first.

ZUSAMMENFASSUNG

Greisen (1972) hat kürzlich auf den „pseudokalorischen Nystagmus aufmerksam gemacht. Die obigen Autoren sind nicht mit den Erklärung der von ihm erhaltenen Resultate einverstanden. Sie schlagen eine andere Erklärung seiner Resultate vor sowie eine Methode, das Phänomen, wenn es auftritt, zu entdecken. Es wird ausserdem gezeigt, dass es nicht ausreicht, den *bithermalen Test auf nur eine Temperatur* zu beschränken, und dass es vorteilhaft ist, die heissen Teste vor den kalten auszuführen.

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Acta Otolaryng 76 183–189 1973

DIE POSITIVE HABITUATION UND DAS VESTIBULÄRE RECRUITMENT

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(Eingegangen am 14. November 1972)

Abstrakt. Im vorliegenden Beitrag soll für die Habituation und das Rekruitment in Bezug auf den Vestibularapparat eine Erklärung versucht werden. Mehrfache rotatorische, thermische, galvanische sowie Pendelstimulationen ziehen keine Habituation nach sich. Der periphere vestibuläre Receptor reagiert nach wiederholten Reizen immer gleich. Habituation ist ein zentraler Vorgang, der bei nervalen und zentralen Läsionen auftritt, unter bestimmten, näher zu erörternden Voraussetzungen wird der Begriff der „positiven Habituation" empfohlen.

Störungen im Cupulaendolymphsystem äußern sich meist infolge wiederholter Stimulationen in einer Zunahme der vestibulären Erregbarkeit, die als vestibuläres Recruitment bezeichnet wird.

Die zahlreichen Untersuchungen mit widersprüchlichen Ergebnissen scheinen zunächst das allgemein bekannte physiologische Phänomen der Habituation, insbesondere die Habituation des Vestibularapparates nicht zu erklären.

Bárány (1967) fand bei verschiedenen vestibulären Reiztechniken keine Habituation so ließ er beispielsweise 14 Tage hindurch täglich 5–10 Drehungen auf einen Patienten einwirken und konnte keine Habituation nachweisen. Für ihn war es jedoch schwer anzunehmen, daß eine sonst generell zu beobachtende neurophysiologische Eigenschaft am Vestibularsystem nicht nachweisbar sein sollte. Er spricht von Gewöhnung, ohne diese objektiviert zu haben. Ebenso fand Jung & Tönnies (1948), offensichtlich in Hinblick auf die Schwindelempfindung, keine wesentliche Reaktionsabnahme nach mehrmaligen Drehungen.

Auch Greiner et al. (1969) konnte nach mehrmaligen Pendelungen in einer Zeitspanne von 2 Minuten bei normalen Versuchspersonen das Phänomen der Habituation nicht nachweisen. Cawthorne (1956) konnte in Untersuchungen an Menschen eine erhebliche Habituation nur bei der Cupulometrie nachweisen. Osterhammel et al. (1968) fand auch bei mehrmaligen, sehr starken Reizen keine Habituation. Fluur et al. (1967) konnte bei wiederholten Untersuchungen mit schwellennahen Reizen eine Zunahme der Latenzzeit beobachten, nicht jedoch bei überschwelligen Reizen. Jongkees (1960) fand im Gegensatz zu Maspetiol et al. (1965) keine Abnahme der maximalen Winkelgeschwindigkeit der langsamen Phase und der Nystagmusfrequenz. Mittermaier (1952) fand bei wiederholten kalorischen Reizungen eine Reaktionsabnahme nur einzelner Nystagmusparameter. Im Gegensatz zu diesen Untersuchungsergebnissen konnten Hazeyama (1963), Henriksson et al. (1961), Brown (1965) und v. Arxt (1964) eine Reaktionsabnahme im Sinne einer Habituation bei Tieren und Menschen feststellen.

Hallpike & Hood (1953) hat vestibuläre Reaktionsabnahmen infolge von Adaptation bzw. Habituation als „response decline" bezeichnet. Fast alle Autoren weisen im Gegensatz zur Nystagmusreaktion eine deutliche Habituation in der Cupulometrie nach.

Tabelle II *Vestibuläres Recruitment*

Die einzelnen Stimulationsmethoden	Kalorische Stimulationen	Rotatorische Stimulationen	Pendelstimulationen
Pendelstimulationen	2	1	0
Rotatorische Stimulationen	22	4	1
Kalorische Stimulationen	2	22	2

Die Häufigkeit des vestibulären Recruitment einzelner Stimulationsmethoden bei *31* Ménièrekranken. (Nur ein Teil der Patienten wurde der Pendelprüfung unterzogen.)

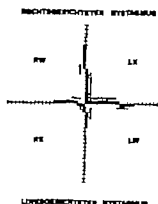


Abb 3. Maria C. 44 a. Acusticusneurinom rechts. Kalorische Werte. Das Pv Vestibulogramm. Positive Habituation. Reaktionsabnahme vorwiegend rechts — 1 Untersuchung. 2. Untersuchung.

bei Akustikusneurinomen eine bemerkenswerte Habituation und eine ziemlich langdauernde Deshabituation (eigene Beobachtung) nachweisen kann möchten wir folgendermaßen erklären Die durch partielle Schädigung der nervalen Elemente entstehenden Impulse und die dadurch bedingte Verschiebung der Ruhepotentiale wirkt für die Habituationszentren so sehr belastend, daß eine weitere Belastung nur mit einer echten Reaktionsabnahme beantwortet werden kann. Ebenso ist die langdauernde Deshabituation durch eine zentrale Dysharmonie bedingt Collins hat durch zahlreiche Untersuchungen ...gewiesen daß die Nystagmusreaktions...me bei wiederholten Stimulationen spe...zifisch ist d. h. daß beispielsweise ein kalorisch habituierter Vestibularapparat normale per und postrotatorische Werte aufweisen kann. Die Befunde von Collins entsprechen denen von Klijn & Ek (1957) sowie Philipszoon (1959).

Habituiert wird ein bestimmtes Reizmuster dabei baut das Zentralnervsystem eine Kopie auf die lange erhalten bleibt und sehr spezifisch ist. Das Cupulaendolymphsystem hat dabei nur eine Transferrolle und beteiligt sich selbst nicht an der Habituation.

Die zentralen Vorgänge bei der Habituation scheinen der Kompensation gleich zu sein. In Intervallen von Stunden und Tagen durchgeführte Vestibularstimulationen bei unilateral ausgeschalteten Patienten rufen eine Hyperreflexie und ein Richtungsüberwiegen zur gesunden Seite hervor Weitere Untersuchungen im Verlauf von Tagen oder Monaten zeigen eine Abnahme des Richtungsüberwiegen bei geringer Verminderung der Hyperreflexie. Subjektiv beschwerdefreie Patienten mit einseitiger vestibulärer Ausschaltung zeigten einen gleichmäßigen Rechts- u. Linksnystagmus, und ein gleichschenkeliges *Kompensationsdreieck* Abb 1 und 2 zeigen den Verlauf des Kompensationsvorganges wobei die dargestellten *Kompensationskurven* und *Dreiecke* die Prognose bestimmen. Diese Ergebnisse fanden wir sowohl bei operativ lädierten Patienten als auch bei Fällen mit Neuronitis Nervi Vestibularis. (Dieses Phänomen kann auch therapeutisch angewendet werden)

Sehr häufig zeigen Patienten mit gestörtem Cupulaendolymphsystem wie Morbus Ménière das Phänomen des *vestibulären Recruitment* Es manifestiert sich bei herabgesetzter Vestibularpotenz in einer Steigerung der Erregbarkeit, letztere kommt in einer Reaktionszunahme bei wiederholt aufeinander folgenden Reizungen zum Ausdruck. In der graphischen Darstellung des Vestibulogramms erkennt man sie an einem Einschwenken der Kurve in den Erregbarkeits-Normbereich. Die Ursache dafür dürfte in einer Labilität des Vestibularapparates liegen. V

Békésy (1939) und später Greiner et al. (1969) konnten bei schwellennaher Stimulierung mit der Torsionsschaukel eine Reaktion nach der einen, nicht hingegen nach der anderen Seite beobachten. Greiner spricht die Vermutung aus, daß es sich dabei um ein vestibuläres Recruitment handelt. Wir sind allerdings der Ansicht, daß primär eine herabgesetzte Erregbarkeit vorliegen muß, denn nur dann wäre ein Recruitment im Sinne eines Ausgleiches der vestibulären Erregbarkeit in Richtung des Normbereiches sinnvoll.

Bei 35 Ménière-Kranken konnten wir in 31 Fällen ein vestibuläres Recruitment beobachten, jedoch war dieses nicht bei allen Stimulationsarten nachweisbar. *Tabelle II* zeigt dessen Häufigkeit bei 31 Ménière-Kranken, nach verschiedenen Stimulationsarten. Greiner konnte dieses Phänomen bei 5% seiner Fälle nachweisen, bei uns liegt diese Zahl wesentlich höher. Diese Diskrepanz scheint darin begründet, daß wir für die Bestimmung des vestibulären Recruitment, nicht nur die Pendelprüfung sondern auch andere Stimulationsmethoden wie Drehung und Kalorisation angewandt haben. Wie aus Tabelle II zu entnehmen ist, muß das Recruitment nicht bei allen Stimulationsformen auftreten bzw. ist es gerade nach Pendelstimulationen relativ selten.

Zur Bestimmung der *vestibulären Habituation* bzw. des *vestibulären Recruitments* wenden wir die monaurale Kalorisation mit konstanter Temperatur an. Dabei wird ein Labyrinth oftmals in Zeitintervallen von 3 Minuten stimuliert. Die Ergebnisse werden in Form der *Vestibularpotenz* errechnet und in ein Kreuzschema eingetragen. Wenn nach mehrmaligen kalorischen Stimulationen bei bestehender Untererregbarkeit keine Habituation oder ein vestibuläres Recruitment nachweisbar ist, wird ein weiterer Test mit dem Dreh- oder Pendelstuhl nötig. Der Rotationstest wird bei uns so durchgeführt, daß jüngere Patienten mit 0,5 /sek² und ältere bis alte Patienten mit 0,9 /sek² bis auf die Endgeschwindigkeit von 100 /sek² acceleriert und nach 10 Sekunden Drehdauer abgebremst werden. Die per- und postrotatorischen Werte werden ebenfalls nach der Formel GA/T errechnet und auf das Kreuzschema eingetragen. Die Ergebnisse der kalorischen Stimulationen der linken Seite, sowie der per- und postrotatorischen Werte nach der Drehung zur linken Seite werden im Kreuzschema rechts, die Ergebnisse der Stimulationen der rechten Seite links eingezeichnet. Der Rechts- bzw. Linksnystagmus werden auf der Ordinate oberhalb bzw. unterhalb der Abszisse eingetragen.

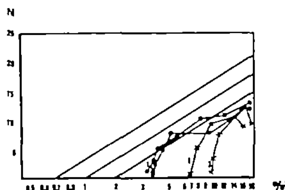


Abb. 5. Gertrude K. 46 a. Acusticusneurinom links. Pendelstimulation. Das Frequenzvestibulogramm. Positive Habituation. Zweimalige Pendelung. Bei beiden Untersuchungen ist eine erhöhte Reizschwelle zu bemerken (links [ ] stärker als rechts [•]). Die positive Habituation ergibt sich aus einer Erhöhung der Reizschwelle nach der zweiten Pendelung.

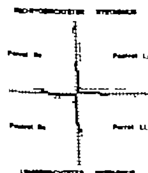


Abb. 4. Aurelia N. 61 a. M. Ménière links. Rotatorische Stimulation. Das Pv Vestibulogramm. Das vestibulare Recruitment beiderseits, overwiegend links manifestiert sich nach der zweiten Stimulation. — 1. Untersuchung. ... Untersuchung

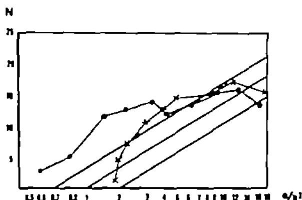


Abb 6 Helarich G 40 a. M Ménière rechts. Pendel stimulation. Das Frequenzvestibulogramm. Vestibuläres Recruitment. Bei stärkerer und mittlerer Reizintensität liegt die Rechtsnystagmuskurve im Normbereich (●). Bei niederer Reizintensität ist eine deutlich herabgesetzte Reizschwelle zu erkennen.

Die Abb 3 und 4 zeigen eine *positive Habituation* bzw ein vest. *Recruitment* nach mehrmaligen thermischen und rotatorischen Stimulationen.

Die Bestimmung der Reaktionsänderungen nach Pendelung ist ebenfalls sehr zuverlässig. Eine zweite Pendelprüfung und Auswertung des Vestibulogrammes nach Frequenz (Greiner et al. 1969) oder nach der *Vestibularpotenz* (Emami Nouri, 1972) erlaubt meistens eine sehr gute Beurteilung der Reaktions-derung. Die Abb 5 und 6 zeigen das Fre-Vestibulogramm bei Habituation und ment.

Mehrmalige galvanische Stimulationen ergeben meist eine gleiche Reaktion weshalb uns diese Methode für die Bestimmung der positiven Habituation oder des vest. Recruitment, nicht geeignet erscheint.

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Wir verstehen darunter „Abnahme der *Vestibularpotenz nach mehrmaligen adäquaten* Reizen ohne Mitwirkung der *Interferenzfaktoren.*"

Das vestibuläre Recruitment kann als Erscheinungsform einer labyrinthär-vestibulären Störung betrachtet werden. Daraus ergeben sich brauchbare klinische Konsequenzen für die Differenzierung zentral-nervaler und labyrinthärer vest. Störungen.

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In this presentation an attempt is made to elucidate the effect of habituation and recruitment on the vestibulo-bulbar system. Repetitive rotation, thermic, galvanic or swinging stimuli, do not cause habituation in the peripheral vestibular system but can be demonstrated in certain neural and CNS lesions. In the author's opinion, habituation is a central process. The term "positive habituation" is recommended. Disturbances in the cupulo-endolymphatic system which are called vestibular recruitment cause upon repetitive stimulation increased excitability of the vestibular systems.

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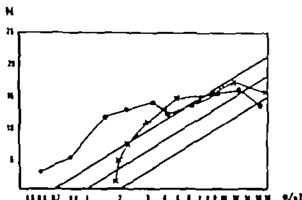


Abb 6 Heinrich G 40 a. M Ménière rechts. Pendelstimulation. Das Frequenzvestibulogramm. Vestibuläres Recruitment. Bei stärkerer und mittlerer Reizintensität liegt die Rechtsnystagmuskurve im Normbereich (●). Bei niederer Reizintensität ist eine deutlich herabgesetzte Reizschwelle zu erkennen.

Die Abb 3 und 4 zeigen eine *positive Habituation* bzw ein vest. *Recruitment* nach mehrmaligen thermischen und rotatorischen Stimulationen

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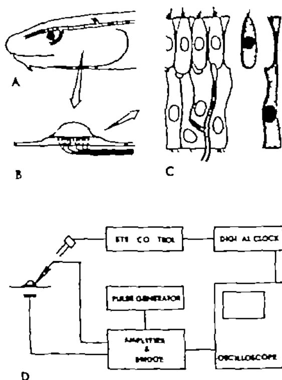


Fig. 1 (A) Diagram of head of burbot, showing the arrangement of lateral line canals and location of organ being investigated in the supra temporal canal. (B) Enlarged view of the organ with cupula supported by sensory epithelium. The branch of the lateral line nerve supplies the organ from beneath. (C) "Exploded" cross-section through sensory epithelium. Flask-shaped hair cells occupy the upper regions of the sensory epithelium, supporting cells extend the entire depth of the epithelium to the basement membrane. Fibres of lateral line nerve retain their myelin sheath some distance above basement membrane. (D) Block diagram of apparatus used to measure electrical properties of lateral line cells.

ultrasonic treatment, first in abs. alcohol then in abs. methanol. The electrodes were filled by capillary action (Tasaki et al. 1968). When filled with 3 M KCl their resistances were 20–60 MΩ and their tip potentials <4 mV. For histological identification electrodes were filled with a 6% solution of Procion Navy Blue H3RS in distilled water (Stretton & Kravitz, 1968). These had resistances of 100–400 MΩ measured in saline and tip potentials of 2–8 mV. Dye was electrophoretically injected by currents of 5×10^{-8}A for 3–15 sec and observed with a microscope. The sensory epithelium was fixed and left overnight in 6% glutaraldehyde buffered at pH 4 with acetate buffer (Kaneko & Hashimoto, 1967). After rapid dehydration in alcohol the specimens were embedded in Epon and polymerized overnight at 60 C. 4–5 μm sections were cut on a serial microtome with glass knives, and examined in a Zeiss photo microscope equipped with differential interference contrast (Nomarski optics).

Electrodes were driven into the cells by means of a micromanupulator provided with a step-motor (Transvertex). The electrode could be rapidly advanced in increments of 2 μm at intervals of 5 or 10 msec and the motion could be triggered by pulses from a digital clock.

Recording

The electrodes were connected to a high input impedance amplifier provided with capacitance neutralization (Mentor), and with a facility for passing depolarizing or hyperpolarizing currents through the electrode. The voltage drop across the electrode resistance could be balanced out in a bridge circuit. Impedance was tested by a continuous train of 5–8 x 10^{-10}A of depolarizing current pulses with a duration of 5 msec and an interval of 5 msec. Stronger current causes polarization of electrodes filled with procion blue.

RESULT

Impedance and membrane potential

Fig. 2 shows three typical recordings from penetrated cells. The recordings on the left of the figure are membrane potentials recorded on a slow time base, to the right is the output of the single electrode bridge recorded on a fast time base. The bridge is balanced just outside the cell. When the cell is penetrated, two events are observed: initially there is a sudden voltage drop which is a measure of the cell membrane potential and secondly the bridge becomes unbalanced. The voltage pulses that develop are a meas-

Acta Otolaryng 76 190–198 1973

PASSIVE ELECTRICAL PROPERTIES OF HAIR CELLS AND SUPPORTING CELLS IN THE LATERAL LINE CANAL ORGAN

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(Received October 9 1972)

Abstract Intracellular membrane potentials and resistance measurements were made from cells in the lateral line organs of the burbot (*Lota lota*). The membrane potentials varied from 10–65 mV and the resistances from 8 110 MΩ A relationship exists between the membrane potential and resistance Under resting conditions there is no detectable difference in the membrane potential and resistance between hair cells and supporting cells. Hair cells are electrically inexcitable and the specific resistance of the hair cell membrane is 100–1 000 Ω/cm and its capacitance is <0.3 μF/cm²

The function of hair cells in the inner ear as a transducer of mechanical into electrical energy is thought to rely on processes related o those which give rise to electrical events nerve cells (Davis, 1965) As a basis for derstanding the process of transduction in air cells it is therefore desirable to know their electrical properties, such as their resting potential, membrane resistance time constant and excitability Because of technical difficulties no report has hitherto appeared on direct measurement of electrical properties of single cells in the inner ear We have used glass microelectrodes to obtain intracellular measurements of these parameters from single cells in the lateral line canal organ which is related to the sense organs of the inner ear Canal organs have hair cells similar to those of the inner ear (Flock, 1965) with the advantage that they are accessible to penetration with microelectrodes.

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METHOD

Preparation

Measurements were made on lateral line canal organs on the isolated head of the freshwater fish *Lota lota* (burbot) and the organ chosen was always the same one (Fig 1) The sense organ was exposed by opening the roof of the canal immediately above it. In isolated head experiments the organ receives no blood supply therefore control measurements were made on the living fish in which the blood supply to the canal organ was intact. They were made spinal, decerebrate and immobilized by Flaxedil (3 mg/kg). Oxygenation was provided by tap water flowing through a mouth piece over the gills, and the head was held rigid in a clamp. All experiments were made at room temperature 20 C.

Electrodes

Electrodes were pulled by a horizontal two-stage puller from Corning Glass (Code 7740) with an outer diameter of 2 mm and an inner diameter of 1 6 mm. Before pulling, one or two glass fibres were inserted into the glass tubes which had previously been cleaned by

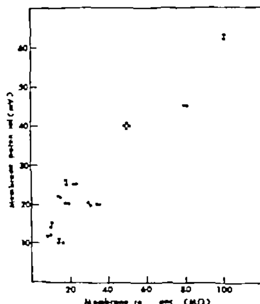


Fig. 5. Relationship between membrane potential and resistance of 60 unidentified lateral line cells.

In an attempt to investigate whether hair cells differed from supporting cells in their resistance and membrane potential, cells were marked by dye injection and identified by histological methods (Fig. 4).

Of 18 marked cells in 13 organs, 14 cells were located in sections and identified as being 7 hair cells and 7 supporting cells. Their resistance, plotted against membrane potential in Fig. 5 shows no obvious difference in the resistance of the two cell types. If hair cells and supporting cells have different impedance or membrane potentials or differ in their susceptibility to damage, then this difference is not large enough to be detected with the present method.

In cell C of Fig. 4 a second barrier was penetrated spontaneously 40 msec after the electrode was advanced into the cell. This second compartment had a potential about 10 mV more negative than the first but did not add any significant resistance (Fig. 4 D). During electrophoretic dye injection a very minute but distinct dot became visible at the electrode tip. Subsequent histological sectioning showed that the nucleus was intensely stained (Fig. 4 C). Loewenstein & Kanno (1963) have found that the nucleus of salivary gland cells is about 13 mV more negative than surrounding cytoplasm and Lindemann & Rikmenspoel (1971) find that bull spermatozoa nuclei have a negative potential of about −6 mV which they attribute to fixed negative charges of the material in the head rather than to selective distribution of mobile ions. We have often observed that in a cell which has been filled with procion dye the dye tends to redistribute so that after 10–30 minutes the nucleus becomes more intensely stained than the cytoplasm. This means that the nuclear membrane is permeable to ions with a relatively high molecular weight which agrees with the lack of a resistance increase as the electrode entered the nucleus of cell C of Fig. 4.

Electrical excitability

Electrically excitable cells change their resistance as well as membrane potential when they are depolarized by current. It is important to know whether the cells of the sensory epithelium have such properties, not only for the phenomenon *per se* but also because the current pulses injected into the cells in our experiments were in the depolarizing direction and would cause an error if these cells were electrically excitable.

We first insured that the cell impedance remained the same for depolarizing and for hyperpolarizing current pulses. The electrodes did not polarize at the current values used. A second way of testing for electrical excitability was to balance the bridge inside the cell and then to see if a superimposed steady depolarizing current of 6×10^{-10}A caused bridge unbalance or voltage change. This amount of current was sufficient to shift the membrane potential about ±15 mV. The result of such current injection is illustrated in Fig. 6. None of the cells tested changed its resistance more than 0.5–1 MΩ (3–6% of their resistance) or its voltage more than 1 mV which is within the limits of error.

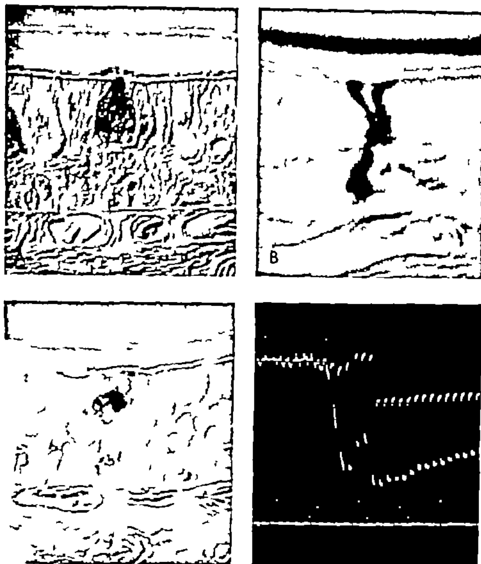


Fig 4 (A) A hair cell and (B) a supporting cell stained by dye injection. (C) The nucleus of a hair cell has been penetrated and stained the record of this penetration is seen in (D) All photographs have been obtained by interference contrast.

Time constant

The time constant (τ) of single cells was determined in the following way With the electrode tip just outside a cell, care was taken to obtain the best frequency response by correct capacity neutralization. KCl electrodes were used because dye electrodes have a poor frequency response. This meant that the cells were not identified but we know from the marking experiments that about 50% of penetrated cells are hair cells. The bridge was balanced outside the cell (Fig 7) The transients seen at the beginning and the end of a current pulse are due to that portion of electrode capacitance which is not compensated for by the recording system, it amounted to less than 0.2 msec. When the electrode penetrated a cell the bridge was balanced to the new zero point. Should the cell membrane add significant capacitance, this would be seen as an increase in the time taken to charge and discharge. Text-fig. 6 shows a cell which did not contribute any significant capacity beyond that of the re-

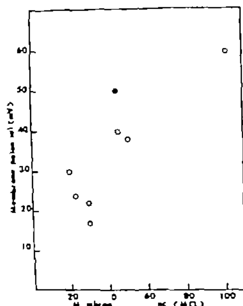


Fig. 5. Relationship between membrane potential and resistance of 7 identified hair cells ( ) and 7 identified supporting cells ( ).

cording system the τ of this cell and others investigated was therefore better than 0.2 msec. A hair cell with a surface area of 1 000 μm^2 an input resistance of 50 MΩ and τ of <0.2 msec would have a specific membrane capacitance of about <0.3 $\mu F/cm^2$

DISCUSSION

When microelectrodes have been advanced through the organ of Corti in the mammalian cochlea negative potentials of as much as −80 mV have been encountered by several authors. It has been suggested that they represent intracellular potentials from cells within the organ of Corti (Békésy 1960, p. 652, Tasaki et al., 1954 Konishi & Kelsey 1968). Others maintain that negative extracellular potentials exist within the organ of Corti since they can be recorded with electrodes as large as 15 μm (Tasaki & Spyropoulos, 1959 Butler 1965) or when the electrode moves as much as 100 μm (Lawrence, 1967). In deaf guinea pigs, cats, and dogs, having essentially no organ of Corti potentials of similar appearance have been described (Tasaki & Spyropoulos, 1959 Suga & Hattler 1970). The origin of this potential has not been established by marking techniques and it has been suggested to be an injury potential which is not present in the normal preparation (Dallos, 1968 Sohmer et al. 1971).

Hair cells, and supporting cells, are small cells and special electrode techniques are required to obtain measurements from them. Iontophoretic dye injection shows that the negative potentials reported here in the lateral line canal organ emanate intracellularly from receptor cells and supporting cells within the sensory epithelium. Their membrane potentials can be as large as −65 mV but are usually closer to −30 mV These are low values compared with neurons and muscle and may well be caused by damage, however the cells are still viable because they produce a receptor potential when mechanically stimulated (Harris et al. 1970 Flock, 1971) and they respond with an inhibitory postsynaptic potential when the efferent nerve fibres which innervate them are stimulated (Flock & Russell, 1972).

Recent findings indicate that rods and cones of the vertebrate retina have a high conductance in the dark which keeps the cell depolarized light reduces this conductance and hyperpolarizes the cell (Toyoda et al. 1969 Baylor & Fuortes, 1970) Since hair cells in the lateral line organ are capable of responding with a hyperpolarization as well as a depolarization when the sensory hairs are bent a similar mechanism has to be considered. The apical cell membrane which surrounds the sensory hairs comprises roughly 10% of the total cell surface and is exposed to the canal lymph which contains 40 mM potassium. A high external potassium environment is known to depolarize cells.

Our measurements of cell membrane resistance have been made with a single electrode bridge balancing system. When the electrode enters the cell its resistance changes and this is indicated by an unbalancing of

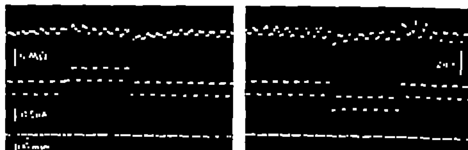


Fig. 6. Effect of depolarizing (*left record*) and hyperpolarizing currents (*right*) on membrane resistance of a hair cell. In each record the lower trace is the time scale, the middle trace is a monitor of the bridge current pulses and the upper trace is the intracellular record.

the bridge. This resistance change has two major components, one due to the resistance of the cell membrane, and the other due to the high specific resistance of the cytoplasm (Tomita & Kaneko 1965 Schanne 1969 Firth & De Felice 1971 Engel et al., 1972). With techniques we used it is not possible to measure the contribution of the second component. However we believe that the largest fraction of the measured cell resistance is due to membrane resistance, since we have observed resistance changes of about 5 MΩ during stimulation of lateral line efferent nerve fibres (Flock & Russell 1972) This possible source of error can be ex...d if a second electrode is placed in the ... cell and current/voltage measurements ... made, but technical difficulties have so far prevented this from being accomplished in lateral line hair cells.

On the basis of resistance measurements in the cochlear partition, Johnstone et al. (1966) calculated that hair cells in the organ of Corti have an impedance of about 48 MΩ Our measurements indicate that hair cells in the canal organ have an input impedance somewhere between 10 and 100 MΩ. The surface area of a hair cell is about 1 000 μm^2 which gives a specific membrane resistivity of 100–1 000 Ω/cm^2 which is somewhat lower but of the same order as that found in other excitable cells (Katz, 1966 Barrett & Crill 1971). Much lower input impedance would be expected if electrical coupling existed within the sensory epithelium (Kaneko, 1971) as might be expected in the goldfish macula sacculi where supporting cells are connected by gap junctions (Hama, 1969). No such junctions have been seen in the canal organ of the burbot (Flock, 1965)

Excitable cells are known to change their resistance as a consequence of a change in their membrane potential. This does not happen in hair cells when their membrane potential is displaced ±15 mV by current. The receptor potential that develops in hair cells during mechanical stimulation barely exceeds ±1 mV therefore electrical excitability *should not be involved to any large extent* in its generation. This finding was not to be presupposed because lateral line canal or

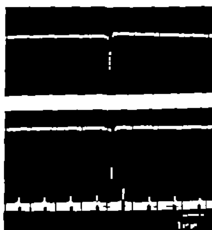


Fig. 7. Response of an electrode to a current pulse injected through it. In the upper trace the electrode has been compensated for resistance and capacity extracellularly. The second trace is intracellular when the added resistance of the cell has been compensated for.

gans are closely related to electroreceptor organs which are extremely sensitive to small electrical fields (Lissmann, 1958 Bennett, 1971).

It is generally supposed that mechanical stimulation of the hair cell excites electrical current flow through the cell which acts on the synaptic mechanism at the base of the hair cell. For this mechanism to be effective at high frequencies of sound it is required that the τ of the cell is sufficiently short. A τ of 0.2 msec means that the cell will not start to attenuate electrical signals until the kHz range is reached, whereas it is known that mechanical damping becomes important above 100 Hz, and is therefore a limiting factor in the lateral line canal organ.

ACKNOWLEDGMENTS

This work has been supported by grants from the Swedish Medical Research Council (B71 14X 2461 04C), the King Gustaf V Memorial Fund and the Funds of the Karolinska Institute.

J Mørup Jørgensen was supported by the Danish Research Council. Ian J Russell was supported by a European Program Research Fellowship from the Royal Society and a fellowship from Magdalene College, Cambridge. We thank Dr S. Shaw for valuable criticism, Mrs Carola Olsén, Hugo Broström and Tore Engberg for skilful technical assistance.

ZUSAMMENFASSUNG

In Zellen der Seitenlinienorgane der Quappe (*Lota lota*) wurden Zellmembranpotentiale gemessen und Widerstandsmessungen durchgeführt Die Membranpotentiale wechselten von 10 bis 65 mV während der Widerstände von 8 bis 110 MΩ änderten Es besteht eine Beziehung zwischen Membranpotential und Widerstand. Im Ruhezustand wurde kein merkbarer Unterschied des Membranpotentials und des Widerstandes zwischen Haarzellen und Stützzellen festgestellt. Haarzellen sind elektrisch nicht reizbar ihr spezifischer Widerstand liegt zwischen 100 und 1 000 Ω cm² und ihre Kapazität beträgt < 0.3 μF cm²

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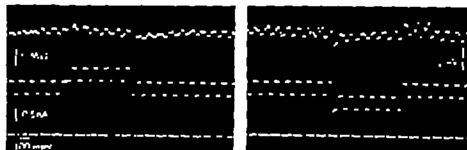


Fig. 6. Effect of depolarizing (*left record*) and hyperpolarizing currents (*right*) on membrane resistance of a hair cell. In each record the lower trace is the time scale, the middle trace is a monitor of the bridge current pulses and the upper trace is the intracellular record.

the bridge. This resistance change has two major components, one due to the resistance of the cell membrane, and the other due to the high specific resistance of the cytoplasm (Tomita & Kaneko, 1965 Schanne 1969 Firth & De Felice 1971 Engel et al. 1972). With techniques we used it is not possible to measure the contribution of the second component. However we believe that the largest fraction of the measured cell resistance is due to membrane resistance since we have observed resistance changes of about 5 MΩ during stimulation of lateral line efferent nerve fibres (Flock & Russell, 1972). This possible source of error can be excluded if a second electrode is placed in the same cell and current/voltage measurements are made but technical difficulties have so far prevented this from being accomplished in lateral line hair cells.

On the basis of resistance measurements in the cochlear partition Johnstone et al. (1966) calculated that hair cells in the organ of Corti have an impedance of about 48 MΩ. Our measurements indicate that hair cells in the canal organ have an input impedance somewhere between 10 and 100 MΩ. The surface area of a hair cell is about 1 000 μm^2 which gives a specific membrane resistivity of 100–1 000 Ω/cm^2 which is somewhat lower but of the same order as that found in other excitable cells (Katz, 1966 Barrett & Crill, 1971). Much lower input impedance would be expected if electrical coupling existed within the sensory epithelium (Kaneko, 1971), as might be expected in the goldfish macula sacculi where supporting cells are connected by gap junctions (Hama, 1969). No such junctions have been seen in the canal organ of the burbot (Flock, 1965).

Excitable cells are known to change their resistance as a consequence of a change in their membrane potential. This does not happen in hair cells when their membrane potential is displaced ±15 mV by current. The receptor potential that develops in hair cells during mechanical stimulation barely exceeds ±1 mV therefore electrical excitability should not be involved to any large extent in its generation. This finding was not to be presupposed because lateral line canal or

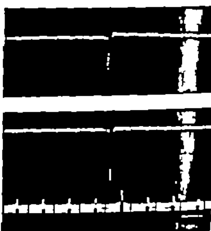


Fig. 7. Response of an electrode to a current pulse injected through it. In the upper trace the electrode has been compensated for resistance and capacity extracellularly. The second trace is intracellular when the added resistance of the cell has been compensated for.

Acta Otolaryng 76: 199-207 1973

THE ULTRASTRUCTURE OF THE AFFERENT SYNAPSE ON HAIR CELLS IN THE FROG LABYRINTH

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(Received December 5 1972)

Abstract. The fine structure of the synaptic contact between sensory cells and afferent nerve terminals of ampullar crista of the frog (*Rana temporaria*) is described. The specializations of this synapse, including synaptic vesicles, and membrane-related structures, show certain differences according to the fixation and contrasting methods used. Parts of these structures are thought to contain proteins which probably are specific for mediating transmitter from the sensory cell to the afferent nerve ending. The mechanism of the synaptic transmission is discussed and thought to be of neuro-chemical type

The hair cells in the inner ear are neuro-epithelial receptors specialized to receive mechanical stimulation. Two types of morphologically distinct nerve terminals are known to innervate hair cells (Engström, 1958 Wersäll et al., 1965) Afferent synapses transmit information from the hair cell towards the central nervous system, and efferent nerve endings are terminals of centrifugal nerve fibers.

The sensory epithelium in the frog's vestibular labyrinth is simple, compared with that of mammals. The hair cells are cylindrical, with an apical bundle of sensory hairs and the nerve endings contact the bottom of the cells (Wersäll et al. 1967) They correspond to the type II hair cells in mammals (Wersäll, 1956). The frog's semicircular canals are innervated by nerve branches which can be easily reached through the otic capsule where they are accessible for studies on nerve terminals and their synaptic relations to hair cells during degeneration and regeneration. Such studies are presented in a separate paper (Gleisner & Wersäll, 1973)

At the sensory synapse between the hair cell and the afferent nerve ending, transmission occurs at specialized synaptic sites. The importance of this synapse as a link in the chain of events that lead to excitation of sensory nerve impulses, is evident and warrants a thorough study of its ultrastructure

MATERIAL AND METHOD

Adult frogs of the species *Rana temporaria* were used. After immobilization with *d*-tubocurarine, the anterior part of the labyrinth was exposed through the palate. Fixation fluid was applied *in vivo* in the perilymphatic space and was also introduced into the endolymphatic space by an incision in the saccule and in one semicircular canal. The anterior and the horizontal ampullae were then dissected out, together with their innervating nerve branches, and placed in the fixative. The fixatives used were osmium tetroxide buffered with Veronal acetate and isotonic with frog plasma (Nilsson, 1964) 3% glutaraldehyde in phosphate buffer (Sabatini et al.

Supported by Grant No. NB 03956-06 National Institutes of Health, the Swedish Medical Research Council (projects No. 12X 720 and 14X 2461) and Therese and Johan Anderssons Minnesfond.

1963), or 1.5% potassiumpermanganate (modified from Luft 1956). After glutaraldehyde fixation the tissue was contrasted with osmium tetroxide or with 1% phosphotungstic acid (PTA) in alcohol (Bloom & Aghajanian, 1966). After dehydration in alcohol the specimens were embedded in Epon and sectioned on an LKB-ultrotome. The sections were usually stained with uranyl acetate and lead citrate (Reynolds, 1963).

RESULT

The sensory nerve fibers loose their myelin sheath before piercing the basal membrane. Non-myelinated peripheral terminals continue their course inside the sensory epithelium where they branch into numerous ramifications which may continue a parallel course for some distance before innervating the bottom of hair cells. The nerve endings may be true terminals of such ramifications, or synaptic contacts may be made *en passant* by passing branches. The sensory synapse is confined to the basal end of the hair cell, nerve endings rarely reach above the level of the nucleus. A survey of the synaptic region is seen in Fig. 1. Each hair cell is generally innervated by more than one afferent nerve ending, apart from its efferent innervation.

In the contact zone, between the hair cell and the afferent nerve ending are specialized synaptic regions characterized by the presence inside the hair cell of a synaptic body surrounded by vesicles and by membrane specializations of the pre- and postsynaptic membranes. This region is regarded as the site where transmission occurs. A number of such sites may exist at each nerve ending (Fig. 2). One and the same nerve ending may have contacts with two hair cells, each contact having a synaptic site (Fig. 3).

Presynaptic region

The hair cell cytoplasm in the synaptic environment contains mitochondria, ribosomes and scattered vesicles. Some vesicles are of the type "coated vesicles". Cytoplasmic tubules sometimes seem to give rise to vesicles. Glycogen granules and filaments are also present.

The presynaptic area is characterized by a spherical structure with an average diameter of 0.3 μm (Fig. 4). The centre of the synaptic body is separated from the presynaptic membrane by a distance of 0.16–0.20 μm. The surface facing the presynaptic membrane is generally somewhat flattened. No delimiting membrane is present between the synaptic body and the cytoplasm.

The appearance of the synaptic body is similar after fixation in osmium tetroxide and after glutaraldehyde fixation followed by contrasting with osmium tetroxide. The matrix appears homogenous with no periodic substructure.

Glutaraldehyde fixation followed by block impregnation with PTA stains the synaptic body in a characteristic manner (Fig. 5). Only the surface layer stains to a depth of approximately 250–500 Å, whereas the central portion remains unstained or only slightly stained. From that surface of the synaptic body which faces the presynaptic membrane slender projections extend and end in bulbous "feet" which closely approximate the presynaptic membrane. Usually no more than three "feet" are seen; they have a somewhat ovoid structure and can also be seen in ordinary osmium fixation.

After staining of the sections with uranyl acetate and lead citrate the interior matrix of the glutaraldehyde-PTA fixed synaptic body is also densely stained. Still, however the contrast of the peripheral margin is enhanced (Fig. 6).

The synaptic body is also seen in tissue fixed in potassium permanganate after staining of the sections with uranyl acetate and lead. Fig. 7 is a schematic drawing intended to show the three-dimensional structure of the synaptic body.

The synaptic body is surrounded by a large number of vesicles about 250–350 Å in dia-



Fig. 1. An afferent nerve fibre (*AF*) is seen to branch and innervate two hair cells (*HC*). On each hair cell two synaptic contact regions are seen (*arrows*).

meter. They surround the synaptic body at a distance of 150–350 Å from its surface. In some sections vesicles seem to be connected to the synaptic body by slender stalks (Fig. 4). Vesicles also occur between the synaptic body and the presynaptic membrane, in this case they are situated between the presynaptic feet. The vesicles have dimensions and an appearance similar to the vesicles which are scattered throughout the basal cytoplasm. They are, however, accumulated in great number in the vicinity of the synaptic body



Fig. 2. Two synaptic bodies in close vicinity to an afferent nerve ending (*ANe*).

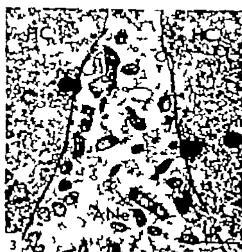


Fig. 3. One afferent nerve ending (*ANe*) may have synaptic contact with two hair cells (*HC*).

The presynaptic vesicles often closely approximate the presynaptic membrane, however no membrane continuity has been observed neither have vesicles been seen in the synaptic space. Coated vesicles which appear in this region are often seen closely adjacent to the presynaptic membrane. Sometimes they originate from this membrane (Fig. 8).









Fig. 4 (a–d). Serial sections through part of an afferent synapse. Osmium tetroxide fixation.

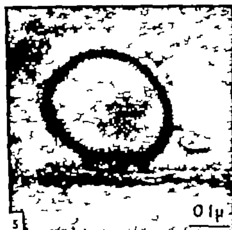


Fig. 5. The synapse is stained in a characteristic manner after glutaraldehyde fixation followed by block impregnation in alcoholic PTA.

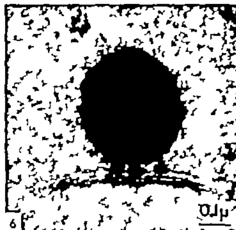


Fig. 6. Uranyl-acetate and lead-citrate stain also the center of the synaptic body and enhances the density of synaptic feet and membrane-associated material. Glutaraldehyde + PTA.

Synaptic membrane specializations

The presynaptic membrane is a continuation of the plasma membrane of the hair cell. In the region adjacent to the synaptic body a waviness of the membrane is sometimes seen, the presynaptic feet being lodged in the depressions (Fig. 6).

The pre- and postsynaptic membranes are separated by a synaptic space of approximately 160–200 Å which in cross section extends for a distance of about 0.5–0.7 μm. At the periphery of the contact zone there is a tendency for the pre- and postsynaptic membranes to come closer together along this border the presynaptic membrane density is increased. In the synaptic space intrasynaptic filaments can be identified. In most sections these filaments are seen to attach to the presynaptic membrane and extend for approximately half the distance of the synaptic space.

In the synaptic space a laminar condensation is characteristically seen as a dense line interposed between the pre- and postsynaptic membranes (Fig. 9). This intracleft substance is also specifically stained by glutaraldehyde PTA which does not stain the plasma membrane. The intracleft substance extends laterally for the same distance as the pre- and postsynaptic membranes.

In ordinary osmium tetroxide fixation as well as in glutaraldehyde PTA preparations, the postsynaptic membrane has an increased density and the staining material appears as a layer of granular substance adhering to the inner surface of the membrane (Fig. 4). It is clearly seen in PTA staining (Figs. 5, 6, 9). The exact relations between the bands of substance stained by PTA in pre-, post- and intrasynaptic zones and the position of pre- and postsynaptic membrane components are difficult to determine. These relations are tentatively indicated in Fig. 10, as suggested by comparative observations from different fixations.

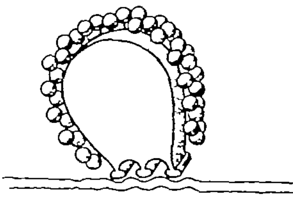


Fig. 7. Schematic drawing of the synaptic body



Fig 8 A coated vesicle (*arrow*) is seen to be open to the intercellular space. Osmium tetroxide fixation.

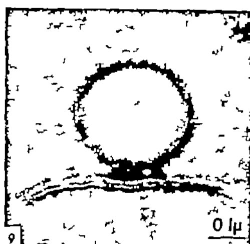


Fig 9 The extension of the membrane associated material and the intracleft substance is well seen after PTA-staining. Glutaraldehyde fixation.

DISCUSSION

The mechanism of nerve impulse initiation in sensory neurons in the eighth nerve has long been debated The vesicles surrounding the synaptic body are similar in dimensions and structure to other synaptic vesicles such as those in muscle motor end-plates and in central synapses. In these cases each synaptic vesicle is known to contain a quantal amount of transmitter substance which upon excitation of the presynaptic terminal is released into the synaptic space to act on the postsynaptic membrane. Here each transmitter package leads to the generation of a miniature postsynaptic potential. Recently Ishii et al. (1971 *a* *b*) have recorded spontaneous and evoked potentials intracellularly from sensory axons in the eighth nerve of the goldfish which they identify as being postsynaptic potentials. Similar potentials have been recorded by Flock et al. (1973) in the non-myelinated portion of the afferent terminal in the lateral line canal organ. This indicates a neurochemical synaptic transmission between hair cells and nerve endings of a quantal nature similar to that in motor end-plates. It has been suggested that the vesicles surrounding the synaptic body represent synaptic vesicles containing a specific transmitter substance of unknown identity which on excitation of the hair cell is released into the synaptic space to excite the sensory nerve ending. The pharmacology of the afferent transmitter is totally unknown the afferent synapse is not blocked by anticholinergic drugs and it fails to stain for acetylcholinesterase (Hilding & Wersäll, 1962 Iurato et al. 1971). The Falck Hillarp fluorescence method for demonstration of catecholamines (1962) is also nega-

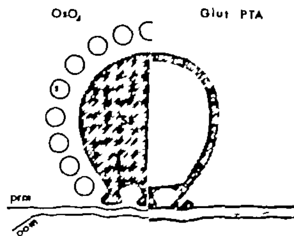


Fig 10 Schematic drawing illustrating how different synaptic structures are stained by osmium tetroxide (*left*) and by PTA following glutaraldehyde fixation (right). *SB* = synaptic body *SV* = synaptic vesicle *prm* = presynaptic membrane, *pom* = postsynaptic membrane.

tive at the base of hair cells (Spoendlin & Lichtensteiger 1967). At the efferent synapse the case is now quite strong for neurochemical transmission. Efferent endings contain vesicles with an appearance similar to those of the afferent synapse, a postsynaptic potential can be recorded intracellularly in hair cells when efferents are stimulated (Flock & Russell, 1972) and pharmacological evidence points towards acetylcholine as being the transmitter (Fex, 1968 Russell, 1971)

In the central nervous system morphological specialization has been described at the synapses between axon terminals and neurons (Gray & Guillery 1966 Akert & Sandri, 1970) Axosomatic and axodendritic synapses have different ultrastructural features and the synapse described in the present work corresponds to the axodendritic type. However at the hair cell afferent synapse a dense presynaptic body is characteristically seen. The synaptic body was first described in the organ of Corti by Smith & Sjöstrand (1961) and similar structures have since been described in most inner ear sensory epithelia in cold blooded animals as well as in mammals (Wersall et al. 1965 Spoendlin, 1965). Analogous specializations are also seen in other sense organs, such as the synaptic ribbon in the eye (Sjostrand, 1958), the synaptic plate in the electric receptor (Barets & Szabo 1962 Mullinger 1964). The presence of this specific presynaptic structure in sensory cells of different sense organs may indicate similarities in the mechanism of sensory synaptic transmission. The close relation between the synaptic vesicles in the hair cell and the synaptic body indicates some functional relationship Similar conclusions are indicated by preliminary experiments by Frishkopf (1972) which show that the diameter of the synaptic body was increased by stimulation of the hair cell in the basilar papilla of the frog. The functional significance of the synaptic body may be related to the charging of the synaptic vesicles with transmitter substance, the mechanism of transmitter release, the synthesis of the transmitter or its function may be a more passive action of guiding vesicle transport to the appropriate presynaptic membrane area. These and other alternatives are, however a matter of speculation.

The specificity of the binding of the PTA to the synaptic body and the pre and postsynaptic membrane related structures has also been observed in the organ of Corti by Nakai & Hilding (1968). This reaction may indicate the presence of proteins rich in basic amino acids as has been discussed by Bloom & Aghajanian (1968) and Bunt (1971) has shown by enzymatic digestion that synaptic ribbons in retinal photoreceptors are composed of proteins.

The presence of ultrastructural specializations in the pre and postsynaptic layer are likely to correlate to molecular arrangements which participate in transmission. The biomolecular changes which occur in the synaptic region in relation to synaptic transmission take place at a macromolecular level which is close to the power of resolution of the electron microscope. Nevertheless the described ultrastructural specializations of the synaptic region must be of functional significance.

Inherent in the synaptic process is the inactivation of transmitter once the appropriate reactions in the postsynaptic membrane are brought about. This process may involve destruction at the postsynaptic membrane. It may also involve the pinocytotic action of coated vesicles associated with the presynaptic membrane as suggested by Wersäll (1968)

The ultrastructure of the sensory synapse in hair cells agrees with the notion that synaptic transmission is neuro-chemical that sensory transmitter is contained in synaptic vesicles in the hair cell, that release occurs in specialized synaptic sites and that multiple sites may contribute to nerve impulse initiation.

ZUSAMMENFASSUNG

Die Feinstruktur des synaptischen Kontaktes zwischen Sinneszellen und afferenten Nervenfortsätzen der Crista ampullaris beim Frosch (Rana temporaria) wird beschrieben. Die Besonderheiten dieser Synapse, einschliesslich der synaptischen Bläschen und membrannahen Strukturen, zeigen nach den verwendeten Fixierungs- und Kontrastierungsmethoden bestimmte Unterschiedsmerkmale. Teile dieser Strukturen enthalten vermutlich Proteine, die als spezifische Mediatorsubstanzen bei der Transformation von der Sinneszelle zum afferenten Nervenfortsatz eine Überträgerrolle spielen. Der Mechanismus der synaptischen Transformation wird erörtert und als neurochemischer Prozess gedeutet.

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Acta Otolaryng 76. 208–220, 1973

DEGENERATIVE PATTERNS IN THE AGING HUMAN VESTIBULAR NEURO-EPITHELIA

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(Received February [illegible], 1973)

Abstract Quantitative analyses of the vestibular hair cell populations from individuals of different ages were performed using the surface specimen technique. There is as much as a 20% reduction of the hair cell populations of the maculae with increasing age. The corresponding figure of the reduction found in the cristae ampullares from old individuals is about 40%. In macula utriculi a pronounced degeneration is found in a small, medial area. Otherwise, the reduction is uniform throughout the macula, as is also the case with macula sacculi. The cristae ampullares exhibit a more pronounced degeneration in a central area on the summit of the cristae than in the periphery. It has been shown by other authors that there is a reduced vestibular excitability in old people. The effect on equilibrium of the vestibular degeneration described in this paper is difficult to establish, there is every reason to believe that there is an …lation between sensory cell loss and reduced …bular excitability

A great deal is still unknown concerning the morphology of the human vestibular end organs. No quantitative studies of the hair cell populations of the vestibular neuroepithelia from individuals of different ages have been carried out so far

v Fieandt & Saxén (1937), Schuknecht (1955 and 1964), Jørgensen (1961 and 1964), Reske Nielsen & Hansen (1964) found normal vestibular hair cell populations in aged individuals.

Krmpotić Nemanić (1964 and 1969) studied the fundus of the internal auditory meatus and found with increasing age a progressive piling up of the bony substance around the foramina through which the nerve bundles pass. She concluded that this apposition of bony substance compressed the nerve fibers, causing presbycusis in the cochlea and, what she called, presbystasis, in the vestibular labyrinth.

Schuknecht et al. (1965) found cochleo-saccular degeneration in an aged cat, an aged dog and one 85-year-old man There was widespread cochlear degeneration in all three cases. They found a loss of about half the number of hair cells in macula sacculi, while the macula utriculi and the cristae appeared normal. They concluded that the phylogenetically younger pars inferior of the labyrinth was more susceptible to aging than the phylogenetically older pars superior

Johnsson (1971) exposed the inner ear by aid of microdissection and studied the innervation of the maculae. He found a degeneration of the innervation of macula sacculi in old individuals. He found only moderate nerve degeneration in macula utriculi. The saccular degeneration was accompanied by loss of statoconia.

Bergström (1973 *a*) counted the nerve fibers of the different branches of the vestibular

Supported by NIH Continuation Training Grant NB-05384-09 1970–71 by the National Aeronautics and Space Administration, NASA Grant NGR 5_-028-003 and by the Swedish Medical Research Council, Project No. B72 1_X 3156-07B.

nerve in man. In aged individuals he found a degeneration of the vestibular nerve with a reduction in number of nerve fibers averaging 37%.

MATERIAL AND METHOD

113 inner ears from 96 individuals were studied. The temporal bones were obtained from 12 fetuses, 9 newborn infants and children, 6 young adults (17 to 40 years old), and 69 middle-aged or old individuals (48 to 95 years old). There was no history of inner ear disease in any of the cases. Ears from individuals treated with ototoxic antibiotics and with cytotoxic drugs were excluded from the study of the hair cell populations of "normals".

The inner ears were in most cases fixed within ten hours after death. 1.5% veronal buffered osmic acid, 4% veronal buffered glutaraldehyde or in some cases, 10% formalin were used as fixatives. In the majority of cases the fixation of the inner ear was performed by a method described by Bredberg (1968).

Those inner ears which were fixed with glutaraldehyde or formalin were later stained with osmic acid. All specimens were rinsed with distilled water after staining and were stored in 70% alcohol. The vestibular sensory regions were exposed by microdissection without previous decalcification of the temporal bone.

The surface specimen technique described by Engström et al. (1962 and 1966) was used for the study. The technique has been modified for application on the vestibular sensory regions (Lindeman 1967 and 1969 *b*; Watanuki et al. 1969; Rosenhall, 1972 *a*). The procedure has been described in detail by Lindeman (1969 *b*) and only its principal features will be recapitulated here. The neuro-epithelium is peeled away from the underlying structures and mounted in glycerine on a glass slide. The specimen is covered by a cover glass, and can be studied with light and phase contrast microscopy. Human specimens stained with osmic acid are often very dark, and light microscopy is usually preferable to phase contrast microscopy. A Wild M 5 stereo microscope and a Wild M 20 light and phase contrast microscope were used. Photomicrographs were taken with both microscopes. Surface measurements were made with a Bausch & Lomb microprojector and with a Wild drawing tube, mounted on the Wild M 20 microscope.

Samples of the hair cell population of the sensory epithelium were counted, using a microscopic eyepiece with a marked field. The density of the hair cells of the sensory epithelium could thus be calculated and the average number of hair cells per 0.01 mm^2 was used to express this density. From this figure and the surface area, the total amount of hair cells of the sensory epithelium could be calculated.

The hair cells of the periphery of each crista ampullaris and those of the central zone on the summit of the crista were counted separately. The total hair cell population of the crista was obtained by adding the figures from the periphery and from the central zone (Lindeman, 1969 *b*; Rosenhall, 1972 *b*). The periphery and the striola of the maculae were usually also counted separately. In many cases the striola appeared very indistinct, and could not clearly be distinguished from the periphery. In those indistinct cases the total hair cell populations were estimated without calculating the different zones separately. Quantitative analyses were obtained from 58 cases.

RESULT

Macula utriculi

There was a moderate but significant ($P <$ 0.001) reduction of the *hair cell population* of the *macula utriculi* of individuals over 70 years of age. Nineteen maculae utriculi from individuals 70 to 95 years of age had a mean hair cell reduction of 21% when compared with the maculae from a control group with "normal" hair cell populations (Fig. 1 and Table I). This control group consisted of fetuses, infants and young adults, and has

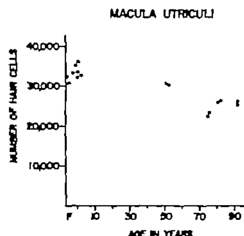


Fig 1 Relationship between total number of hair cells of macula utriculi and age. *F* indicates the fetal material. There is a significant but moderate reduction of the total hair cell population with aging.

been reported earlier (Rosenhall 1972 *a*, *b*). The hair cell populations of the aged maculae varied considerably A few showed no reduction at all others exhibited marked reductions with more than 30% of the hair cells missing.

In order to evaluate regional differences in the degenerative pattern it is advantageous to consider the density of the hair cells in different regions of the macula. When comparing air cell densities between individuals of different ages, the prenatal group was excluded. is is because the fetal end organs are smaller and their hair cells more densely packed than those found in the postnatal group

In the postnatal part of the control group, there were mean values of 78 6 sensory cells/ 0.01 mm² in the periphery of the macula and 63 1 cells/0.01 mm² in the striola. In individuals 70 years or older the corresponding figures were 65 1 and 54.3 cells/0.01 mm² respectively (Table II) The degree of reduction of the hair cell population was about the same in the periphery as in the striola. In the prenatal group the difference in hair cell density between the periphery and the striola was about the same as in the postnatal group. In some fetuses, especially those younger than four months, the border of the macula utriculi was usually somewhat indistinct, and the most marginal zone close to the border had a low hair cell density This lack of distinction of the border and the low hair cell density were observed to be particularly pronounced in the most medial part of the macula (Fig. 2). In all other groups of individuals studied, the macular borders usually appeared distinct. A blunt indentation was occasionally seen in the medial border of the macula utriculi (Fig. 3). In infants and young adults the area close to the indentation had about the same hair cell density as the rest of the periphery of the macula. A concentration of type II cells was found here but no distinct morphological polarization was seen in this area. In old individuals the hair cell density of this medial area was often considerably decreased (Fig. 4 A B) Over the age of 70, there was an average of only 47 1 sensory cells/0.01 mm² found in this area in eight maculae, where this particular area could be evaluated. This figure is about 25% lower than the cell density (65 1) of the periphery of these maculae. A high degree of variation was present. In some maculae this localized degeneration was very pronounced with almost 40% fewer hair cells

Table I. *The mean total number of hair cells of human vestibular sensory epithelia in different age groups*

Age group	Macula utriculi	Macula sacculi	Lateral crista	Superior crista	Posterior crista
Prenatal group–40 years	33 100	18 800	7 600	7 600	7 600
41 70 years	31 800	18 200	6 100	6 200	6 300
71–95 years	26 100	14 200	4 700	4 700	4 300

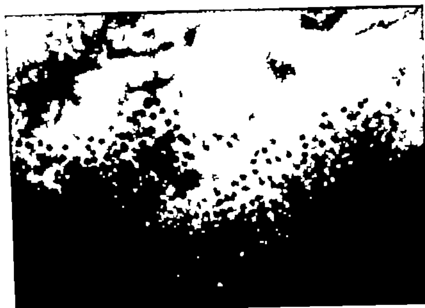


Fig The medial part of macula utriculi from a 4-month fetus. The borders of the sensory epithelium are indistinct, and there is a lower density of the hair cells in the most medial zone. ×190

Fig 3. Macula utriculi from an adult. The striola (S) is seen as a dark stripe in the center of the sensory epithelium. A blunt indentation (*arrow*) is present in the medial part of the macula. *pi*, pars interna, *pe* pars externa. 36

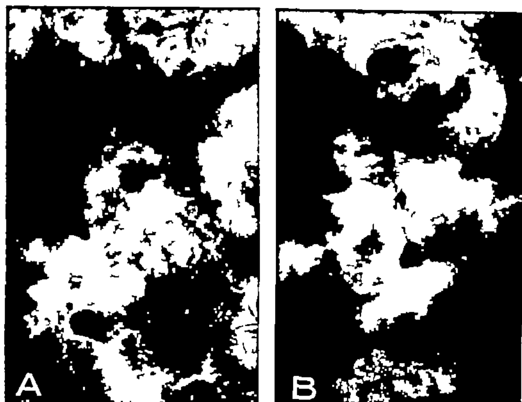


Fig 4 Macula utriculi from a 76-year-old male. (A) Surface preparation from the medial zone of the macula. 1 200. (B) Surface preparation from an adjacent region in the pars interna, outside the striola. 1 200. There is a conspicuous reduction of the hair cell population in the medial zone.

than adjacent areas of the macula. In other maculae this medial area had about the same degree of degeneration as the rest of the macula.

Macula sacculi

The *macula sacculi* showed a moderate, significant reduction in number of hair cells ($P < 0.001$) in individuals 70 years of age or older (Fig. 5 and Table I) The degree of degeneration was about the same as in the macula utriculi. Eighteen maculae sacculi from individuals 70–92 years old exhibited an average total hair cell reduction of 24% compared with the control group having normal vestibular sensory regions. The degree of the reduction was very variable ranging from no significant reduction at all to pronounced degeneration. Usually however the degeneration was only moderate (Fig. 6)

In many cases it was difficult to evaluate separately the periphery and the striola in macula sacculi. In those cases in which this evaluation was possible, the degeneration was uniform all over the macula the degree of the hair cell reduction was about the same in the periphery as in the striola (Table II).

Cristae ampullares

The *cristae ampullares* showed a more pronounced age related reduction of hair cell populations than the maculae (Fig. 7A, B). Such reductions were already distinguishable in many temporal bones of middle-aged individuals, 50–60 years of age The average reduction in number of the hair cells of all three cristae in individuals 70 years or older was about 40% compared to the control group (Fig. 8 and Table I) This significant degeneration ($P < 0.001$) was about the same in all three cristae. In individuals 70 years or older the lateral crista contained an average of 4 700 hair cells (ranging from 3 100 to 5 900), the superior crista 4 700 hair cells (ranging

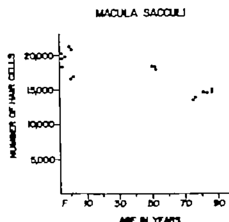


Fig. 5. Relationship between total number of hair cells of macula sacculi and age. The reduction of the hair cell population is about the same as that found in macula utriculi.

from 2 900 to 6 500) and the posterior crista 4 300 hair cells (ranging from 3 000 to 5 600).

Some of the cristae from old individuals had almost normal hair cell populations, but in the oldest individuals, pronounced degenerative changes were found in almost all cases. In some of these specimens up to 60% of the hair cells were missing. Cristae from the same inner ear showed about the same degree of degeneration. However, in some ears, a considerable variation was present.

The loss of hair cells was diffusely spread over the sensory epithelium, though regional differences were present. The loss was more prominent in the central area on the summit than in the peripheral region of the crista.

In the postnatal part of the control group there were averages of 66.5 sensory cells/0.01 mm² in the central zone, and 81.0 cells/0.01 mm² in the peripheral zone. In the group 70 years or older the figures were 35.0 cells/0.01 mm² for the central zone and 55.1 cells/0.01 mm² for the peripheral zone (Table II). In the control group the difference in density between the central and the peripheral zone was 18% (Rosenhall, 1972 *b*) while the corresponding figure was 36% in the group of old individuals, a difference which is statistically significant.

Degenerative patterns of type I and type II hair cells

The present technique is not very suitable for discrimination between type I and type II vestibular hair cells in surface preparations from humans. However, a rough estimation was possible in a few cristae.

In four cristae from different individuals over 80 years of age, attempts were made to estimate the proportions of the two cell types. In all of these cristae 50–60% of the hair cells in the periphery and 70% or a little more in the central zone were of type I. It must be emphasized that these figures are questionable, and other techniques, e.g. electron microscopy, are needed to obtain reliable information in this field.

Comparison of the sensory regions from the same vestibular apparatus

The degree of degeneration usually followed a clear pattern. In most of the cases in the age group 70 years or older, the cristae exhibited a more pronounced degeneration than the maculae. In inner ears with none, or only minute degeneration of the maculae, the de-

Table II. *The mean number of hair cells per 0.01 mm² in different regions of the human vestibular sensory epithelia*

Age group (years)	Macula utriculi		Macula sacculi		Cristae ampullares	
	Periphery	Striola	Periphery	Striola	Periphery	Central area
0–40	78.6	63.1	76.0	62.7	81.0	66.5
41–70	71.9	61.4	75.1	65.1	70.7	53.8
71–95	65.1	54.3	64.6	52.6	55.1	35.0



Fig 6 Surface preparation of macula sacculi from an 89-year-old male. The hair cells appear fairly normal with only moderate signs of age-related degeneration. Only a very slight reduction of the total hair cell population is present in this sensory epithelium. 2 000.

generation of the cristae was more pronounced but yet only moderate. In inner ears with marked macular degeneration the cristae ex hibited still more severe degeneration.

Exceptions from the usual pattern of degeneration

Some exceptions from the normal degenerative pattern were noticed. In two cases (HV 47 and HV 82) the macula sacculi and in another case (HV 68) the macula utriculi exhibited the same degree of degeneration as the cristae.

Another case deviated particularly from what was usually found. This 79-year-old male (HV 40) was known to have bilateral sensori-neural hearing loss one year prior to death. The patient died from cardiac failure. Examination of the left ear showed that the macula sacculi was much smaller than normal, 1.48 mm² compared to 2.44 mm² normally seen. The shape was abnormal with a shallow indentation of the inferior border The superior bulge innervated by Voit s nerve, was reduced in size (Fig. 9) and the borders to the sur

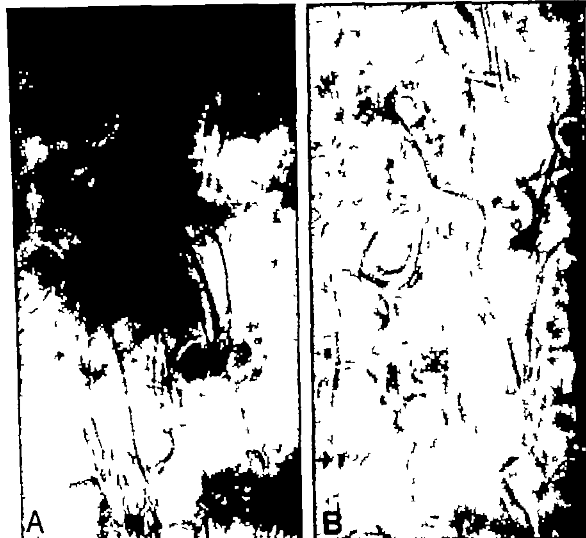


Fig. 7. (A) The periphery of a lateral crista of an 89-year-old woman. There is a pronounced reduction of the number of hair cells. × 1 900. (B) Corresponding area of a lateral crista from a newborn infant. This crista has a full complement of hair cells. × 2 000.

rounding perimacular zone were indistinct in this area. The total hair cell population of the macula was approximately half the value normally found in this age group. The macula utriculi was slightly smaller than normal, and its hair cell population reduced. The cristae showed only very slight reduction of the hair cell populations.

Correlation of clinical and histologic findings

In some cases otoneurological examinations had been performed prior to death. One of the cases, a 55-year-old male (HV 48) with no history of inner ear disease, had an otoneurological examination 2 1/2 years before death. Vestibular tests, with electronystagmography (ENG) and also pure tone audiogram were normal. The patient died of renal failure. Both maculae and two of the cristae from the left ear were examined. The size and form of the neuroepithelia were normal. All hair cell populations were normal and no significant degenerations were discernible.

Another case, a 70-year-old male (HV 57)

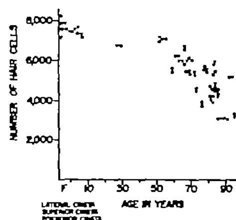


Fig 8 Relationship between total number of hair cells of the cristae ampullares and age. There is a marked reduction of the hair cell populations with age. The reduction, exceeding what is seen in the maculae, is about the same for all three cristae. Superior crista = crista anterior

also had no past history of inner ear disease In 1968 he was given 12.0 g of streptomycin sulfate for pulmonary tuberculosis. An otoneurological examination was performed after the treatment Pure tone audiograms at two different times showed bilateral high frequency sensorineural hearing loss. ENG showed no ntaneous or gaze nystagmus. A weak posi- bal nystagmus was present. Caloric re onses were normal. The ENG was repeated two weeks later and this time showed no positional nystagmus. The caloric responses were again normal. The patient died of intracranial hemorrhage two years later Post mortem examination of the left vestibular apparatus showed neuroepithelia of normal size and form. There was a slight reduction of the hair cell contents of both maculae. The cristae showed reductions of about 25% of the hair cell populations, which is within the common range.

Some additional cases where ENG had been performed have been reported separately An anomaly of the posterior crista has been described (Rosenhall, 1973 *a*) In this case, a 68-year-old woman hair cell reductions of 20–25% were found in the vestibular sensory regions of the left ear ENG was performed prior to death and showed that the left ear responded in a normal fashion to caloric irrigation. However no comparison could be made with the right ear which had failed to respond either to auditory or to caloric stimulations following a presumed cerebrovascular accident. In another paper (Rosenhall, 1973*b*), the vestibular morphology of three cases with caloric hypo-reactivity is described. One of these cases showed a left-sided caloric hyporeactivity five years prior to death. Examination of the left ear showed pronounced degenerative changes within the superior part of the labyrinth.



Fig 9 Survey picture of a macula sacculi with an unusual size and shape. The sensory epithelium is smaller than usually seen. The superior bulge (arrow) is much smaller than normal There is a shallow indentation of the inferior border The cytoarchitecture of the sensory epithelium is normal for the age. This macula probably represents an anatomical variation. ×57

DISCUSSION

In this investigation vestibular neuro-epithelia from individuals of different ages were compared. Inner ears from fetuses, newborn infants and young adults were considered to have normal sensory epithelia. Bredberg (1968) has used a similar approach in comparing individuals of different ages in his study of the human cochlea. He found cochlear hair cell degeneration in young individuals. In the present material no reductions of the number of hair cells in the vestibular regions were discerned before the age of 40. However only very few temporal bones from children and young adults were available for study The number of temporal bones from individuals between 1 and 40 years of age is not large enough to permit the conclusion that no hair cell reductions occur in this age group and it is not possible to tell exactly at what age the reductions of the hair cell populations start.

Degenerative changes of the vestibular end organs have been found in specimens from subjects over the age of 40 The decrease of the number of hair cells is initially insignificant, but over the age of 70 the cristae exhibit marked reduction of hair cell population. Caloric hypo-reactivity in old subjects has been observed (Allard, 1938 Arslan, 1957 Rossberg, 1964 Minnigerode et al. 1967). An increase of the vestibular responsitivity up to the age of 40 (van der Laan, 1972) or up to the age of 60 (Bruner & Norris, 1971) has been reported. In old age the authors mentioned above described vestibular hypo-reactivity which may be due to the marked degeneration of the cristae described here.

Bredberg (1968) showed that in the cochlea severe degeneration of sensory cells and nerve fibers was frequently present in aged individuals. This degeneration was especially localized to the basal and apical turns of the cochlea and was particularly marked in the outer hair cell region. The effects of acoustic trauma have been suggested as an explanation for this degenerative pattern. Comparable severe localized degenerations are not typical for the vestibular sensory regions, which exhibit diffuse degeneration. Morphologically the vestibular end organs and the cochlea exhibit important basic differences, and they apparently react in a different way to acoustic insults. The vestibular apparatus is phylogenetically older than the cochlea and thus probably less vulnerable to damage. The inner hair cell of the cochlea, which is thought to be more resistant to noxious agents than the outer hair cell, shows obvious morphological similarities to the vestibular sensory cell (Engström, 1958 and 1967 Iurato 1962). Another possible explanation for the diverse patterns of degeneration within the labyrinth is the difference in vascular supply between the cochlea and the vestibular end organs.

The degenerative pattern seen in the vestibular end organs is a moderate degeneration of the maculae, and a more pronounced degeneration of the cristae. This corresponds very well with the observations of Bergström (1972 and 1973 *a*) who has found an age related nerve degeneration of totally about the same magnitude.

The degeneration of the sensory epithelia is particularly marked in the central area of the crista, while the macular striola shows about the same degree of degeneration as the rest of the macula. In old subjects Bergström (1973 *b*) found a pronounced rarefaction of the thick, myelinated nerve fibers which supply the summit of the crista.

The present study contradicts the concept that the vestibular sensory structures are not morphologically affected by aging (v Fieandt & Saxén, 1937 Schuknecht, 1955 and 1964 Jørgensen, 1961 and 1964 Reske Nielsen & Hansen, 1964 Naufal & Schuknecht, 1972) Some of the discrepancy between earlier reports and the present one is probably due to the fact that different techniques have been used. Quantitative estimations of sensory cell populations are difficult to obtain with sectioning techniques. Age does not seem to affect the general shape or the thickness of

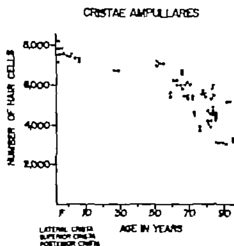


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the sensory epithelia, and they may appear normal in sectioned material.

Schuknecht et al. (1965) Krmpotić Nemanić (1971), and Johnsson (1971) have reported cochleo-saccular degeneration in cases with presbycusis. The present study has not shown any such cases. It must be emphasized, however that the cases in this study were randomly chosen, and no effort was made to select cases with marked presbycusis.

A few cases in this study show a higher degree of macular degeneration than is usually seen and one case exhibited a saccular abnormality The macula sacculi of the latter case was reduced in size, but the neuroepithelium appeared normal for the age This finding probably represents an anatomical variation

In the medial region of the macula utriculi, a blunt indentation (Fig. 3) is occasionally seen. This part of the macula exhibits some qualities that differ from the rest of the macula. The region is developed late in fetal life and it is more susceptible to degenerative changes than the rest of the macula. It also contains more type II cells than the rest of the macula and the hair bundles of this medial region show no distinct morphological polarization. In the guinea pig the indentations of both maculae are prominent, and the hair cell density is lower in the indentated parts (Lindeman, 1969 b Watanuki & Meyer zum Gottesberge 1971 c).

According to this study the patterns of degeneration related to aging resemble the degenerative patterns found after administration of ototoxic antibiotics. Pronounced degeneration is found in the cristae especially in a central area on the summit after administration of ototoxic antibiotics such as streptomycin and kanamycin (Berg, 1951 Hawkins & Lurie, 1952 Igarashi et al. 1966 Lindeman, 1969 a Watanuki & Meyer zum Gottesberge, 1971 b) The maculae also exhibit degeneration but to a lesser degree than the cristae. The same pattern is found in aged individuals. It is possible that the vestibular sensory epithelia react in a similar way to various types of noxious agents. However the degenerative patterns related to aging and to ototoxic drugs are not identical. In aged inner ears both maculae exhibit the same degree of degeneration, and the degeneration is as pronounced in the striola as in the periphery This is not the case after administration of ototoxic drugs, when the macula utriculi shows more damage than the macula sacculi, and the macular striola shows more damage than the periphery (Lindeman, 1969 a).

Streptomycin is especially harmful to the type I hair cell (Wersäll & Hawkins, 1962 Spoendlin, 1966) X-ray irradiation causes more damage to the type II cell (Winther 1969). The findings concerning the destructive effect of aging on the different hair cell types are inconclusive However the present study indicates a similar vulnerability in relation to age (compare Rosenhall, 1972 b) It should also be mentioned that there is considerable controversy concerning the distribution of the cell types in the normal crista. Some authors have reported a concentration of type I cells on the summit of the crista (Wersäll, 1956 Spoendlin 1965 Lim 1971 Rosenhall, 1972 b), while others have found an even distribution of the two cell types (Lindeman, 1969 b Watanuki & Meyer zum Gottesberge 1971 a).

Some of the cases presented here had been submitted to otoneurological testing prior to death. Reductions of the vestibular hair cell populations of up to 25% were found in ... ears from which apparently normal cal... responses had been recorded. One of the ca... had received streptomycin sulfate. The ... tibular sensory regions of the ... morphologically normal consi... age.

ZUSAMM...

Quantitative An... von Individuen in bei Verwendung geführt worden. der Haarzellen sich bis auf 20% ... Reduzierung der ...

ist ungefähr 40%. In Macula utriculi ist in einer kleinen, medialen Fläche eine ausgesprochene Degeneration gefunden worden. Im übrigen ist die Reduzierung über der ganzen Macula gleichförmig verteilt. Dasselbe gilt auch bei der Macula sacculi. Die Cristae ampullares zeigen in einer zentralen Fläche am Gipfel der Cristae eine mehr ausgesprochene Degeneration als in der Peripherie der Cristae. Von anderen Verfassern ist gezeigt worden, dass bei alten Leuten eine Abnahme der vestibularen Empfindlichkeit stattfindet. Die Wirkung der in dieser Schrift beschriebenen vestibularen Degeneration auf das Gleichgewicht ist schwierig festzustellen. Es gibt indessen gute Gründe anzunehmen, dass eine Beziehung zwischen dem Verlust von Haarzellen und der Abnahme der vestibularen Empfindlichkeit vorliegt.

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Acta Otolaryng 76, 221–228, 1973

KARYOTYPIC VARIATION IN BENIGN PLEOMORPHIC ADENOMA OF THE PAROTID AND IN NORMAL SALIVARY GLANDS

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(Received August 30, 1972)

Abstract. Numerical and structural karyotypic variation was found in cells cultured *in vitro* from 6 benign mixed tumours (pleomorphic adenoma) of the human parotid gland. Three submaxillary glands with chronic sialadenitis and 6 histologically normal salivary glands from subjects with neoplasms of the upper aero-digestive tract were studied originally as controls, but found to display a similar degree of karyotypic variation, mainly due to gain and/or loss of −E and −G-like chromosomes. Normal karyotypes were found in the normal salivary glands from 2 subjects with other conditions The hypothesis is put forward that salivary gland tissue is liable to karyotypic variation for the presence of some factor(s) *in vivo* and or its activation *in vitro*.

Numerical and structural chromosome anomalies have been extensively studied in human malignant tumours while very little information is available on the chromosomes of benign tumours.

The purpose of this work was to study the karyotypes of mixed tumours (pleomorphic adenomas) of the parotid gland. These tumours are usually benign (Foote & Frazell, 1954 Eneroth, 1964 Eneroth & Hjertman, 1966 Evans & Cruickshank, 1970) and also in our cases the histology and the clinical behaviour indicated their benign nature. We ound in this material a considerable amount of karyotypic variation. We had planned to use as controls apparently normal salivary glands from patients with chronic sialadenitis and with tumours of other districts of the aero-digestive tract but we found karyotypic variation also in this material and therefore this unexpected result is reported in detail.

MATERIAL AND METHOD

The sex, age, and histological diagnosis of each subject are entered in Tables I to III. None of the subjects had received physical or chemical treatment prior to surgery Immediately after surgical removal, a thin slice of salivary gland tissue was taken for histopathology and the remaining tissue was grown in T flasks with Eagle MEM supplemented with 20% calf-serum. After variable time a monolayer of cells was established and chromosome preparation were made from cells either grown on coverslips or dispersed by trypsinization. The cells after Colcemid treatment (0 05 μg/ml) for 3 hours were exposed to hypotonic treatment (0 95% sodium citrate for 20 min at room temperature), fixed in methanol and glacial acetic acid 3 1 air dried, and stained with acetic orcein. Fluorescence analysis was made after staining with quinacrine mustard according to Caspersson et al. (1971)

RESULT

Pleomorphic adenoma

Histological examination revealed in all the cases the typical appearance of benign pleomorphic adenoma with prevalence of the myxoid component. The results of chromosome analysis of the 6 cases are shown in Table I Each of the six tumours had cells with both normal and deviant karyotypes, in the pro-

Table I. *Pleomorphic adenoma of the parotid re _ of chromosome analysis*

Case	Sex	Age	Cells karyotyped			Number (in parenthesis) and description of abnormal cells				
			Total	Normal	Abnormal	44	45	46	47	48
1	♀	29	5	—	5	—	(4) −F (1) −B	—	—	—
2	♂	25	19	4	15	(1) −F−G	(2) +M_1−C−G (1) +M −C−D (1) +D−F−G	(8) +M_1−C	(1) +G	(1) +M −C+G+G
3	♂	57	28	7	21	(1) −C+F−G−G (1) −C−C+F−G (1) +F−G−G−G (1) −A−C	(1) +M_1−C−D (1) −D (2) −G (2) +F−G−G	(1) +A−C (1) +A−B−C+E (2) +F−G (1) +M_1−E+F−G (1) M_s (1) +C−D	(1) +C (1) +E	(1) +M_1+G
4	♂	32	20	12	8	—	(1) −D	(1) +E−G	(4) +G (1) +E	(1) +F+G
5	♀	31	11	10	1	—	(1) −C−D+E	—	—	—
6	♂	32	43	42	1	—	—	—	(1) M	

One cell with 90 chromosomes and +A−B−D−E−E+G

Table II *Chronic sialadenitis of the submaxillary gland results of chromosome analysis*

Case	Sex	Age	Cell karyotyped			Number and description of abnormal cells		
			Total	Normal	Abnormal	45	46	47
1	♂	64	25	6	19	(15) −G	(1) +C−G	(2) +G (1) −C+D+F
2	♂	53	13	9	4	(2) −G	(1) +C−G	(1) +B
3	♀	47	10	7	3	(1) −C (1) −B	—	(1) +G

portions entered in Table I Most deviations were numerical, but one characteristic marker chromosome was repeatedly found in cases 2 and 3 and two other markers in one cell each of cases 3 and 6 Deviations in chromosome numbers involved more frequently chromosomes of groups C, F and G

Case 1 Only 5 cells were karyotyped but all had 45 chromosomes and in 4 of them the missing chromosome was one of the F group.

Case 2 Eight of the 15 abnormal cells had 46 chromosomes but the consistent presence of a long acrocentric chromosome (M_1) with a length comparable to that of one of the shorter chromosomes of the C group, and the absence of a C group chromosome. A likely interpretation of the M_1 chromosome is that it originated by a pericentric inversion of a C chromosome. The same M_1 chromosome was present in 2 cells with 45 chromosomes and in one cell with 48 chromosomes, and also these 3 cells had a missing C chromosome One blood culture from this patient revealed only normal cells.

Case 3 Three of the 21 deviant cells had a new chromosome apparently similar to M_1 the other ones having only numerical deviations, most of them produced not only by chromosome loss but also by the presence of extra chromosomes.

Case 4 Five of the 8 abnormal cells had 47 chromosomes and 4 of these were +G

Case 5 One cell out of 11 was abnormal having 45 chromosomes through the loss of one C and one D and the presence of one extra E chromosome.

Case 6. This was analysed with the fluorescence technique Forty two cells were normal and one cell had an extra chromosome which would have been labelled as −E like by the conventional techniques. This chromosome had a banding pattern different from any normal one and must have originated by structural rearrangement(s) that remained unidentified.

Chronic sialadenitis

Submaxillary glands of 3 patients with clinical and histological diagnosis of chronic sialadenitis were cultured originally as controls. However in at least 2 of these cases the numerical deviations found were far more numerous than those expected on the assumption of random deviations (Table II). Thus, in Case 1 15 of the 19 abnormal cells were consistently 45 XY −G and in Case 2, 3 cells were also −G

Salivary glands from patients with tumours of the upper aero-digestive tract

Four parotid and 3 submaxillary glands, all apparently normal, also intended as controls, were obtained from 7 patients with malignant or benign tumours of the upper aero-digestive tract or of the parotid as specified in Table III.

Case 1 The analysis of 10 metaphases from the histologically normal salivary tissue of this patient with papillary cystoadenoma lymphomatosum of the parotid revealed 5 normal cells one cell with a marker similar to M_1 associated with the absence of one C and one G chromosomes, and 4 cells with loss and or gain of F and G chromosomes.

Case 2 A similar situation was found in cultures of submaxillary gland from the patient with an epithelioma of the tongue. In fact, of the 15 cells examined, only 4 had a normal complement. Among the abnormal ones, one showed the M_1 long acrocentric marker and a missing chromosome in C group 5 cells with 45 chromosomes had the loss of G and E chromosomes and each of 4 cells had consistently one extra F chromosome.

Cases 3 and 4 In these 2 cases with epithelioma of the larynx the majority of cells from the parotid tissue were abnormal. In case 3 14 of the 29 abnormal cells were 45 −G and 3 were 47 +F while in case 4 there were two 45 −G and two 47 +G cells.

Case 5 In cultures from the submaxillary

Table III *Histologically normal salivary glan _ om subjects with different types of tumours of the upper aero-digestive tract results of chromosome analysis*

Case	Sex	Age	Cell karyotyped			Number and description of abnormal cells						Gland studied	Histological diagnosis of tumour
			Total	Normal	Abnormal	44	45	46	47	48	>48		
1	♂	52	10	5	5	—	(1) +M −C−G (1) −F (1) −G	(1) +F−G	(1) +G	—	—	Parotid	Papillary cystoadenoma lymphomatosum
2	♂	76	15	4	11	—	(2) −E (3) −G	(1) +M −C (1) +C−G	(4) +F	—	—	Submaxillary	Epithelioma of the tongue
3	♂	50	34	5	29	(1) −C−F (1) −C−G	(14) −G (2) −C	(1) +C−G	(3) +F (1) +C (1) +C+C−G	(1)+G (1)+E (1) +C+F	(1) +C+C+C 1 (94) +C+F	Parotid	Epithelioma of the larynx
4	♂	49	6	1	5	—	(1) −E (2) −G	—	(2) +G	—	—	Parotid	Epithelioma of the larynx
5	♂	54	20	2	18	—	(6) −G	(2) $+M_1$−C−E+G (1) T(+Bq −Cq) (1) −D−D+C+G	(4) +G (2) +E (1) +M	—	—	Submaxillary	Epithelioma of the floor of the mouth
6	♂	47	8	1	7	—	(1) +D C−G (1) +C−G−E (1) −G	(5) +C−G	—	—	—	Submaxillary	Epithelioma of the larynx
7	♀	28	14	14	—	—	—	—	—	—	—	Parotid	Pleomorphic adenoma of the parotid

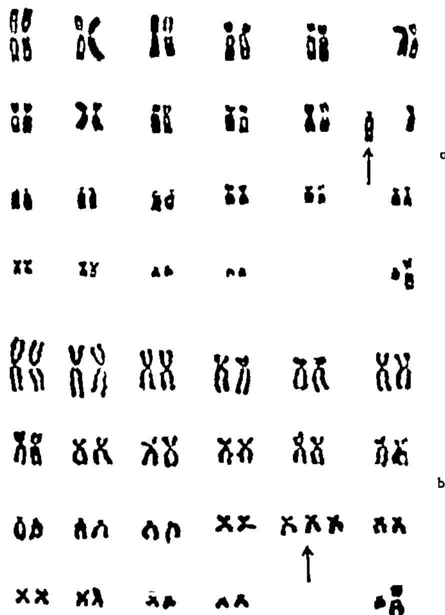


Fig. 1 (*a*) Case 2 of Table I. Metaphase with 46 chromosomes, marker chromosome (M_1, arrow) and one C chromosome missing. (*b*) Case 4 of Table I. Metaphase with 47 chromosomes. Extra E-like chromosome (arrow).

gland there was a prevalence of numerical abnormalities of G chromosomes, 6 of the 18 abnormal cells being 45 −G and four 47 +G. In this case there was also 2 cells with the M_1 marker and +C +G −E.

Case 6. Five of the 7 abnormal cells from the cultured submaxillary gland tissue were consistently 46, +C, −G. One blood culture from this case revealed only normal cells.

Case 7. All the 14 metaphases from the histologically normal portion of the parotid of this patient with benign pleomorphic adenoma, showed normal karyotypes.

In conclusion, also in these apparently nor

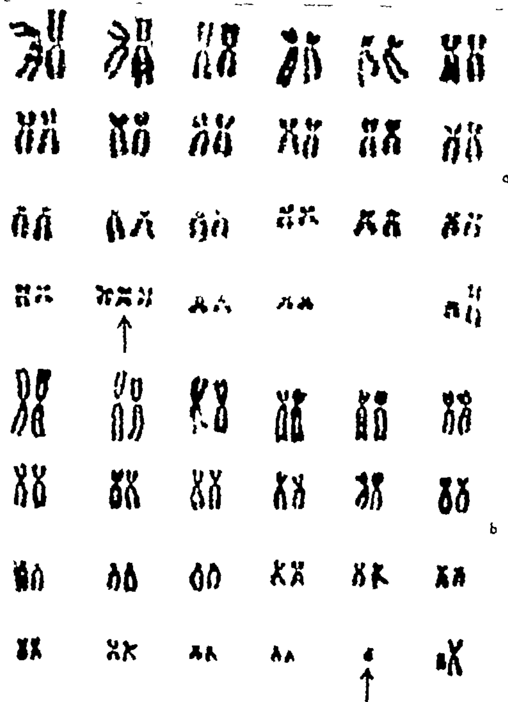


Fig. 2. (*a*) Case 3 of Table III. Metaphase with 47 chromosomes. Extra F-like chromosome (*arrow*). (*b*) Case 5 of Table III. Metaphase with 47 chromosomes. Extra G-like chromosome (*arrow*).

mal salivary gland tissues from 2 patients with benign and 5 with malignant tumours there were a high proportion of chromosomal abnormalities, the most frequent variations being the gain and/or loss of G and F chromosomes.

Two normal submaxillary glands from other sources

Macroscopically and histologically normal submaxillary glands were obtained from a 72 year-old man who died of acute heart failure and from a 43-year-old man operated be-

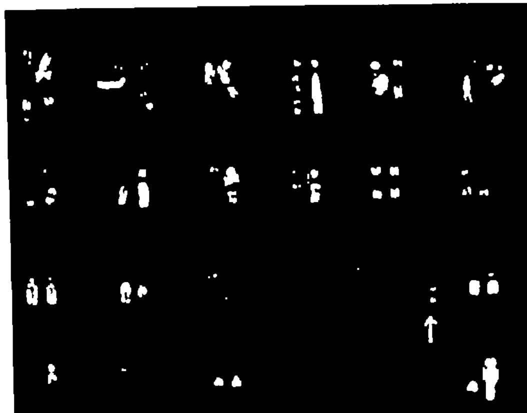


Fig. 3 Metaphase with 47 chromosomes stained with quinacrine mustard. Extra chromosome (*arrow*) has fluorescence bands different from any other chromosomes. All other chromosomes have normal bands.

cause of neuralgia of the lingual nerve. In each case 7 cells were analysed in detail and showed a normal karyotype.

DISCUSSION

The finding of numerical and structural chromosome abnormalities in the pleomorphic adenomas of the parotid gland in absence of any histological sign of malignant transformation confirms the scarce but indicative evidence that karyotypic variation is present also in benign tumours. Thus, Zang & Singer (1967), Mark (1969) and Benedict et al. (1970) reported chromosomal abnormalities in benign intracranial tumours, while Fraccaro et al. (1968) found correlation between signs of initial malignant transformation and clonal evolution of one abnormal karyotype in a cystic adenoma of the ovary Conversely as a complete surprise came the finding of essentially the same type of chromosomal variation including the presence of a marker chromosome probably due to a pericentric inversion of a C chromosome and a prevalence of numerical variation in defect and excess of −E and −G-like chromosome, in a series of normal salivary glands from subjects with benign and malignant tumours of other districts and with chronic sialadenitis. The only exception was the finding of normal karyotypes in the histologically normal section of parotid adjacent to a pleomorphic adenoma (case 7 Table III). The only other normal chromosome picture was found in the 2 submaxillary glands from the subjects with heart failure and neuralgia of the lingual nerve.

We are satisfied that our findings are not due to technical artefacts. In fact, we have

carefully controlled the large number of primary cell cultures from different tissues of various subjects which have been made concurrently in our laboratory and found no evidence of karyotypic variation of the extension and type found in the salivary glands. One could then postulate that salivary glands *in vitro* are more liable than other tissues to numerical and structural chromosome aberration. These aberrations could be due either to some product(s) of these cells in culture or to presence in these cells *in vivo* of some factors, for example viruses, able to induce chromosomal aberration. If this were true we should find karyotypic variation in *all* cultured salivary glands and for this reason we need to examine more glands like those of the last 2 controls, which were found normal. In this respect, we have cultured 2 normal tonsils (another organ known to be liable to virus storage) and found no evidence of karyotypic variation

An alternative hypothesis is that the karyotypic variation found in the normal glands from subjects with tumours and sialoadenitis is directly or indirectly produced by some unknown factor(s) connected with the presence of the abnormal condition itself With one exception (case 6 Table I) we performed our analyses when the new techniques for chromosome identification were not yet available We should be able in the near future to define most of the karyotypic variation and reveal any eventual consistent pattern in it.

ACKNOWLEDGMENTS

We thank Professors M. Cherubino, M Fraccaro and Dr L. Tiepolo for their constant interest in this work. We acknowledge with gratitude the help of Dr U Magrini with the histological analyses.

ZUSAMMENFASSUNG

Numerische und strukturale karyotypische Anomalien wurden in Zellen die „in vitro" kultiviert waren und von 6 gutartigen, gemischten Tumoren (pleomorphischen Adenoma) die von der menschlichen Ohrspeicheldrüse stammten beobachtet. Ursprünglich zu Kontrollzwecken wurden 3 Unterkieferdrüsen mit chronischer Sialoadenitis und 6 histologisch normale Speicheldrüsen von Patienten mit Neoplasmen des oberen aerodigestiven Teils untersucht. Auch in diesen Fällen wurde eine karyotypische Variation gefunden, die in der Hauptsache von einer Überzahl und/oder dem Verlust von E und G-ähnlichen Chromosomen herrührt. Normale Karyotypen wurden in Kulturen von Patienten mit abweichendem Zustand beobachtet. Hypothetisch wird angenommen, dass das Gewebe der Speicheldrüsen wegen des Vorhandenseins gewisser Faktoren und deren Aktivierung „in vitro" karyotypischen Anomalien unterliegt.

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Acta Otolaryng 76. 229–238, 1973

THE VESTIBULAR RESPONSE FOLLOWING UNILATERAL VESTIBULAR NEURECTOMY

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(Received January 19 1973)

Abstract. 15 patients suffering from unilateral Menière's disease were investigated before and at regular intervals for 3 years following vestibular neurectomy The spontaneous vestibular signs as well as the caloric response of both labyrinths were recorded by electronystagmography The process of vestibular compensation occurring after the excision of Scarpa's ganglion presented with two phases: (1) An acute phase (lasting for 4 weeks after surgery) and (2) a chronic phase (lasting from the first postoperative month to the third postoperative year).

The acute phase of compensation was characterized by the rapid but incomplete regeneration of the resting activity of the homolateral vestibular nuclei and by a strong central inhibition of the contralateral vestibular nuclei. During the chronic phase of compensation the reduction of the central inhibition was accompanied by an increased activity (recruitment) of the homolateral vestibular nuclei during caloric stimulation. Because of the different postoperative behaviour of the vestibular system at rest (spontaneous vestibular signs) and during caloric stimulation, the compensatory process occurring following vestibular neurectomy has to be considered under a static and a dynamic aspect. Only the evolution in time of the dynamic compensation—and not that of the static compensation—was found to correlate with the subjective postoperative complaints of the patients. The dynamic aspect of the vestibular compensation may therefore be used to objectivate the imbalance experienced by the patient following vestibular neurectomy

Vestibular neurectomy has proven most successful in eliminating vertigo while preserving hearing in patients suffering from Menière's disease (Fisch, 1970). The operation, which is performed through the middle cranial fossa, consists in the excision of the meatal segment of the vestibular nerve. Since Scarpa's ganglion is included in the resected specimen, vestibular neurectomy offers the ideal condition to study the compensatory process taking place in the vestibular system following the unilateral elimination of the first vestibular neuron in man.

METHOD

Fifteen patients suffering from unilateral Menière's disease were investigated before and at regular intervals for 3 years following vestibular neurectomy The average age of the patients was 47 ± 19 years. The spontaneous vestibular signs as well as the caloric response of both labyrinths were recorded by electronystagmography (Fisch, 1965 Fisch & Siegfried, 1965). The caloric stimulation was performed with the irrigation of 20 cc of water of 27 C, respectively 47 C for 20 seconds. The following parameters were selected for study: (*a*) the frequency and average velocity of the slow component of the spontaneous and positional nystagmus, (*b*) the duration of the caloric response, and (*c*) the average velocity of the slow component and the frequency of the nystagmus measured over a period of 10 seconds of the maximal intensity of the caloric response (Reichold, 1969).

RESULT

Spontaneous Vestibular Signs

(*a*) *Spontaneous nystagmus.* All patients presented after surgery with a spontaneous nys-



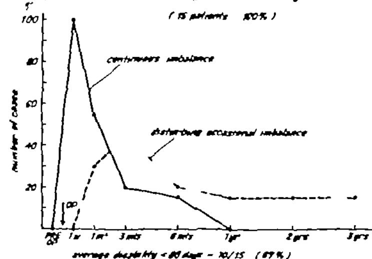


Fig. 1 (A) Progression of the intensity of the spontaneous nystagmus following vestibular neurectomy (B) Progression of the subjective sensations of imbalance experienced by the patient following vestibular neurectomy 100% = 15 patients.

gmus directed towards the intact ear (Fig. 1 A) The intensity of the spontaneous nystagmus, as measured by the average velocity of the slow component, was maximal in the first postoperative week and decreased rapidly thereafter reaching a value of $\bar{x}=5$ /sec ($s=1°$/sec) which was kept practically constant until the end of the observation period of 3 years. A similar pattern was found for the frequency of the spontaneous nystagmus which reached the average of $\bar{x}=18$ beats per 10 sec ($s=3$) at the end of the first week and a constant value of $\bar{x}=5$ beats per 10 sec ($s=2$) thereafter

(*b*) *Positional nystagmus.* A positional nystagmus was also present after vestibular neurectomy and always directed towards the intact, contralateral ear until the end of the third postoperative year The evolution of the intensity of the observed positional nystagmus was identical to that of the spontaneous nystagmus.

The postoperative course of the spontaneous vestibular signs imply that, in spite of a rapid initial compensation a permanent imbalance of the vestibular tonus in favour of the contralateral ear persists for at least 3 years following the excision of Scarpa's ganglion The intensity of the spontaneous vestibular signs is not a good measure of the subjective sensation of disequilibrium experienced by the patients. In fact, despite already stabilized minimal spontaneous vestibular signs, quite a number of patients com-

plain about disturbing imbalance after the first postoperative month (Fig. 1 B).

Caloric Response

No specific caloric reaction was obtained after warm or cold water irrigation of the homolateral ear (operated side) in all 15 patients. For this reason only the preoperative and postoperative caloric response of the contralateral ear was used for further evaluation. The normal average parameters of the caloric reaction have been established by examining 30 patients with healthy ears between 20–30 years of age (average age 25 years).

Duration

(*a*) *Total duration.* The sum of the response to warm and cold irrigation of the intact side is defined as the total duration of the caloric reaction. After excision of Scarpa's ganglion this parameter is reduced in average to 60% of the preoperative value after 1 week, to 85% of the same value at the end of the first and during the following 11 months (Fig. 2 A) and reaches its preoperative value at the end of the third postoperative year.

The observed reduction of the total caloric response of the non-operated side after vestibular neurectomy can be explained by a long-lasting *central inhibitory effect*. The central inhibition has a transitory maximal peak in the first 4 postoperative weeks (acute phase of compensation) and persists, although with decreasing intensity in the remaining 3 years (chronic phase of compensation).

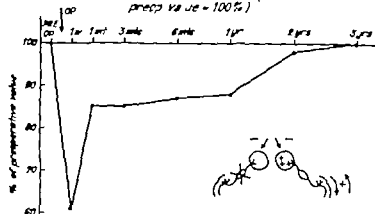
(*b*) *Relative duration.* By plotting the average duration of the single warm or cold water response of the contralateral side as a percentage of the corresponding total duration of the caloric reaction it is possible to analyse the postoperative relationship (prevalence) between the responses to each thermal stimulus (Fig. 2 B). The warm water reaction dominates in the postoperative phase with a peak at the end of the first postoperative week and is progressively reduced in intensity thereafter. The relative duration of the cold water response is, conversely, reduced to the half of the preoperative value initially (first postoperative week), recovering slowly thereafter without, however, reaching its original value even at the end of the third postoperative year. According to Fluur (1960, 1961) the response to cold (resp. warm) irrigation is dependent upon the level of the spontaneous activity present in the homolateral (respectively contralateral) vestibular nuclei. The postoperative evolution of the relative duration of the cold water response can, therefore, be considered as proportional to the level of the resting discharge present in the homolateral, deafferented vestibular nuclei. This activity is sharply reduced to less than half during the first postoperative week, then recovers rapidly until the end of the first postoperative month and very slowly (chronic phase) subsequently without reaching the original, preoperative value, even at the end of the third postoperative year.

Maximal intensity

(*a*) *Total maximal velocity of slow component.* This parameter is defined as the sum of the average velocity of the slow component measured during the maximal intensity of the caloric response to warm and cold water. The total maximal velocity of the slow component is reduced, on average to 65–75% of the preoperative value for as long as 6 months after surgery (Fig. 3A). Later-on, the preoperative value is reached and even exceeded at the end of the third postoperative year.

A central inhibition followed by a subsequent facilitation has to be accounted for the observed changes of the total maximal velocity of the slow component after excision of Scarpa's ganglion. The inhibitory effect presents an initial peak (acute phase), and then decreases slowly in intensity over a period of one year or more. The postoperative variations of the maximal velocity of the slow component of the warm and cold water nys-

A Total duration (warm and cold water response, preop value = 100%)



B Relative duration of the warm and cold water response (total duration = 100%)

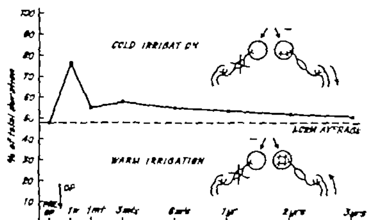
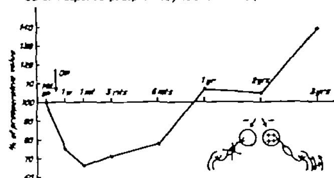


Fig. 2 Duration of the caloric response of the intact, contralateral ear following vestibular neurectomy (A) The sum of the response to warm and cold irrigation (total duration) is given as a percentage of the preoperative value. The curve represents the average for the 15 patients. The schematic representation of the vestibular end-organs, vestibular nerve and vestibular nuclei should demonstrate how the depression of the peripheral activity of the vestibular system by cold water irrigation facilitates the contralateral activity and how augmentation by warm water irrigation inhibits it. (B) The relative duration of the warm and cold water response is represented as a percentage of the corresponding total duration. The normal average for warm water irrigation has been found to be $\bar{x} = 48\%$ ($s = 7.5$). This means that in a healthy subject there is a slight prevalence of the response to cold irrigation. The represented values for the relative duration of the warm and cold water response are the average of all 15 patients.

tagmus were analysed separately in relation to the corresponding total values. The data obtained (Fig. 3 B) are very similar to those established for the relative duration of the caloric response The caloric nystagmus directed towards the contralateral side (response to warm irrigation) prevails, particularly at the end of the first postoperative week (acute phase) and is then slowly reduced in its intensity over the following 3 years. The average preoperative value, which corresponds to a predominance of the caloric nystagmus directed towards the homolateral, operated side (irritative lesion due to Menière's disease) is never reached again At the end of the second postoperative year however a temporary reversal of the predominance of the nystagmus from the contralateral (response to warm irrigation) to the homolateral side (response to cold irrigation) is recorded. This



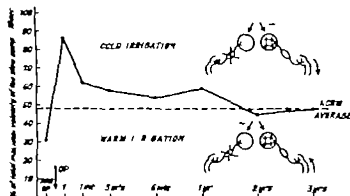
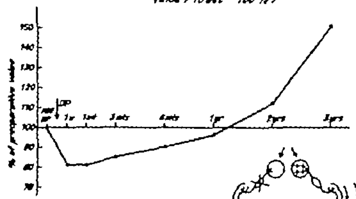


Fig. 3. Maximal intensity of the caloric response of the intact ear following vestibular neurectomy. (A) The sum of the average velocity of the slow component for cold and warm irrigation is given in per cent of the preoperative value. The curve represents the average obtained for all 15 patients. (B) The average maximal velocity of the slow component for the warm irrigation is represented as a percentage of the corresponding total maximal velocity. Each value of the relative average velocity of the slow component is an average of all 15 patients. The normal average for the relative velocity of the slow component of the warm caloric response was found to be: $\bar{x} = 48\%$ $(s = 10.5\%)$.

temporary predominance of the maximal cold water response occurs at a time in which the spontaneous vestibular signs are still present and directed towards the opposite side. If the discrepancy between the direction of the spontaneous nystagmus and that of the prevalent maximal caloric nystagmus is explained in terms of different activity levels in the vestibular nuclei, one has to admit that in spite of a still insufficient resting discharge, the vestibular nuclei of the operated side are able to increase their activity over the normal average level during the maximal intensity of the caloric response. This behaviour could be defined as a recruitment or over recruitment of activity occurring during maximal stimulation in the deafferented vestibular nuclei.

(c) *Total maximal frequency.* The sum of the frequency of the cold and warm water nystagmus measured at its maximal intensity shows very similar changes to those observed for the total maximal velocity (Fig. 4 A). An average reduction of activity to 80% of the preoperative value occurs during the first postoperative year and is later followed by



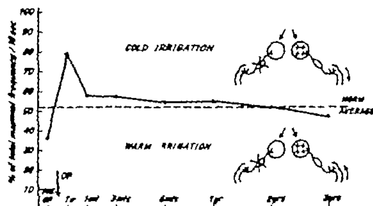


Fig. 4 Maximal intensity of the caloric response of the intact ear following vestibular neurectomy (A) The total maximal frequency (sum of the cold and warm water response) is given as a percentage of preoperative value The curve is an average of 15 patients. (B) The relative maximal frequency of the warm irrigation is given in percent of the corresponding total maximal frequency The normal average for the relative maximal frequency of the warm water response in normal subjects has been found to be: $\bar{x} = 52\%$ ($s = 8\%$).

a rebound of activity reaching 150% of the original value at the end of 3 years. These figures confirm the time evolution of the central inhibition of the contralateral vestibular nuclei as already determined by the total maximal velocity of the slow component.

(*d*) *Relative maximal frequency* The relationship between the maximal frequency of the cold and warm water nystagmus (Fig. 4 B) corresponds closely to that obtained for the maximal velocity of the slow component (Fig. 3 B). The postoperative prevalence of the caloric nystagmus towards the contralateral side (response to warm irrigation) shows a peak with a rapid reduction at the end of the first postoperative month, followed by a slow decrease during the next 11 months. Later-on a reversal of the prevalence of the caloric nystagmus from the contralateral to the homolateral side is recorded. This reversal, which takes place in spite of a spontaneous nystagmus still beating in opposite direction, can be explained again by a recruitment of activity occurring in the homolateral vestibular nuclei during maximal caloric reaction.

DISCUSSION

The compensatory process taking place in the vestibular system following unilateral vestibu-

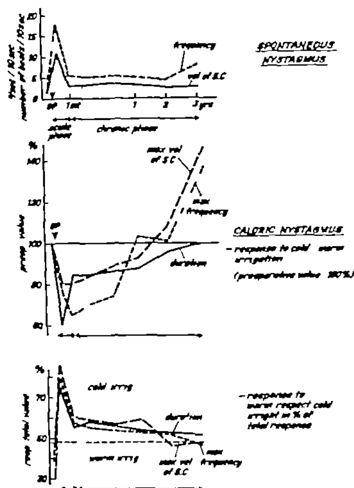
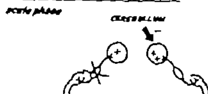


Fig. 5. Labyrinthine function following unilateral vestibular neurectomy. The time evolution of the spontaneous nystagmus and of the caloric nystagmus is shown in order to demonstrate the acute and chronic phase of vestibular compensation. The postoperative compensation of the spontaneous vestibular sign is subtotal and already achieved at the end of the first postoperative month. In spite of the stabilized spontaneous and positional nystagmus (static compensation) the caloric nystagmus shows a continuous evolution during the total observation period of 3 years (dynamic compensation).

lar neurectomy shows a different evolution in time if measured according to the changes of the spontaneous vestibular signs or to those of the caloric reaction of the contralateral ear (Fig. 5). The postoperative spontaneous and positional nystagmus is already reduced to a minimum 4 weeks after surgery. In contrast to this the duration and intensity of the caloric nystagmus show a slower post operative modification lasting for as long as 3 years. Because of this difference, the process of vestibular compensation occurring after the excision of Scarpa's ganglion can be divided into two phases: 1) an *acute* phase (lasting for 4 weeks after surgery), and 2) a *chronic* phase (lasting from 1 month to 3 years after surgery).

The acute phase of the vestibular compensation is characterized by the onset and rapid reduction of the spontaneous vestibular signs. During the acute phase of compensation the vestibular nuclei of the operated side quickly restore their spontaneous activity though without regaining it entirely. The result of





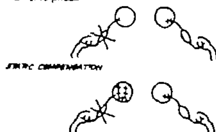


Fig 6 Schematic representation of the process of vestibular compensation following unilateral vestibular neurectomy The acute phase is characterized by a strong central inhibition and by the loss and incomplete regeneration of the resting activity of the deafferented vestibular nuclei. During the chronic phase a static and dynamic compensation should be distinguished. The static compensation is expressed by constant spontaneous vestibular signs indicating that the resting activity of the homolateral vestibular nuclei has not reached the preoperative value and that, therefore a continuous imbalance of the vestibular tonus persists at rest. The dynamic compensation is characterized by a recruitment of activity in the homolateral vestibular nuclei during the maximal intensity of the caloric response.

this incomplete recovery is a permanent imbalance of the vestibular tonus, which is minimal and is only detected with the aid of electronystagmography The rapid subtotal compensation of the spontaneous vestibular sign does not correlate with the course of the subjective sensations experienced by the patients after the operation (Fig. 1 B) This is understandable since these sensations are mainly related to movement while the spontaneous vestibular signs are expression of the *static* and not of the *dynamic* condition of the vestibular system.

The dynamic behaviour of the vestibular system was investigated by the caloric stimulation of the contralateral side since no specific caloric response was observed after surgery from the homolateral (operated) side. The analysis of the *duration* and *maximal intensity* of the caloric response of the contralateral ear revealed that the acute phase of compensation (particularly reflected by the spontaneous and positional nystagmus) is followed by a longer lasting *chronic* compensatory process (*chronic* phase of compensation). The chronic phase of compensation affects the dynamic response of the vestibular system in two ways: 1) by a *central inhibition* acting over the contralateral vestibular nuclei, and 2) by a *recruitment of activity* in the vestibular nuclei of the operated side. These two compensatory processes are expressed by the reduction of the total duration, total maximal intensity and frequency of the caloric response of the intact ear (Figs. 2 A, 3 A, 4 A, and 5) as well as by the evolution of the directional preponderance of the caloric nystagmus (Figs. 2 B 3 B, 4 B, and 5)

The central inhibitory effect observed after excision of Scarpa s ganglion in man corresponds to that observed recently by McCabe et al. (1972) after labyrinthectomy in the cat. These authors write that: "In response to a massive asymmetry of discharges into the cerebellum occasioned by the sudden deafferentation of the vestibular nuclei of one side (by unilateral labyrinthectomy) cerebellar inhibition imposes a shutdown of the intact side, to rebalance the asymmetry at the lowest level" According to the present findings the central inhibition is always inversely proportional to the level of the resting activity present in the vestibular nuclei of the operated side and is therefore at its strongest when the resting discharge of the vestibular nuclei is at its minimum and vice versa. The very intense and prolonged central inhibition of

the vestibular nuclei occurring after vestibular neurectomy may explain why after surgery the patient is recovering from his vestibular loss in a few days and is able to leave the hospital on his own at the end of the first postoperative week.

The time evolution of the process of vestibular compensation occurring after excision of Scarpa's ganglion has been summarized in Fig. 6. An *acute* phase of compensation occurs in the first 4 postoperative weeks. During this time a strong *contralateral central inhibition* is present and the *resting activity* of the homolateral vestibular nuclei is rapidly but incompletely *regenerated*. The acute phase of compensation is followed by a chronic compensatory process during which the central inhibition is slowly reduced and the deafferented vestibular nuclei show a constant activity at rest, but an increasing activity during maximal caloric stimulation. The difference between resting activity of the homolateral vestibular nuclei as determined by the spontaneous vestibular signs and by the caloric stimulation makes it necessary to distinguish between *static compensation* and a *dynamic compensation*. The fact that a recruitment of activity occurs in the deafferented vestibular nuclei during maximal caloric stimulation explains why the prevailing direction of the maximal caloric nystagmus may be opposite to that of the spontaneous vestibular signs between the second and third postoperative year.

The time evolution of the dynamic compensation is in good accordance with that of the complaints of the patient about subjective postoperative imbalance (Fig. 1B). The dynamic compensation is in fact nearly completed 1 year after surgery at a time when the subjective complaints of the patient about imbalance are also reduced to a minimum.

Three patients out of 15 continued to complain about disturbing occasional imbalance 1 year after surgery. Two of them had significant evidence of incomplete dynamic compensation (significantly abnormal values for the maximal intensity of the caloric response of the intact ear). The third patient with an average static and dynamic compensation lacked any personal ambition or motivation to resume work and was tending towards an invalidity claim from his insurance. It is hoped therefore that the knowledge of the dynamic process of vestibular compensation will give some objective clues to measure the validity of the subjective complaints of the patient following vestibular neurectomy.

ACKNOWLEDGMENT

The technical assistance of Miss L. Somm and Mrs L. Regeler are thankfully acknowledged.

ZUSAMMENFASSUNG

15 Patienten mit einer einseitigen Ménièreschen Erkrankung wurden bis zu 3 Jahren nach einer Vestibularisneurektomie *untersucht*. Die spontanen vestibulären Zeichen sowie die kalorische Reaktion beider Labyrinthe wurden elektronystagmographisch aufgezeichnet. Die Kompensation des durch die Exzision des Ganglion Scarpae hervorgerufenen vestibulären Ausfalles verlief in zwei Phasen. Eine erste, akute Phase von 4 Wochen Dauer wurde von einer zweiten, chronischen Phase abgelöst, die bis zum 3. postoperativen Jahr andauerte. Die akute Phase der vestibulären Kompensation zeichnete sich durch die rasche wenn auch unvollständige Regeneration der Ruheaktivität der homolateralen sowie durch die starke zentrale Inhibition der kontralateralen vestibulären Kerne aus. Die chronische Phase der Kompensation wurde von einer Veränderung der zentralen Inhibition und von einer Zunahme der Aktivität (Recruitment) der homolateralen vestibulären Kerne während der kalorischen Reizung begleitet. Die nach einer Vestibularisneurektomie einsetzende Kompensation muss infolge ihres unterschiedlichen Verhaltens in Ruhe (*spontane vestibuläre Zeichen*) und nach kalorischer Reizung unter einem statischen und einem dynamischen Aspekt betrachtet werden. Die postoperativen, subjektiven Gleichgewichtsstörungen der Patienten korrelierten mit dem zeitlichen Ablauf der dynamischen und nicht mit dem der statischen Kompensation. Die dynamische Kompensation kann somit als objektives Mass für die durch die Exzision des Ganglion Scarpae hervorgerufenen, subjektiven vestibulären Beschwerden verwendet werden.

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Acta Otolaryng 76 239–243 1973

OPTOKINETIC NYSTAGMUS IN ACOUSTIC NEUROMAS

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(Received January 17 1973)

Abstract. In 17 patients with operatively confirmed, mostly large acoustic neuromas, optokinetic nystagmus was analysed relative to the size of the tumour and its relation to the brain stem. In 6 patients optokinetic nystagmus was abnormal, and in these cases the tumour was large or very large, in most cases adherent to the brain stem. Abnormal optokinetic nystagmus is due to pressure by the tumour upon the pontine optokinetic centre. In 11 patients optokinetic nystagmus was normal; in 5 of these patients the tumour was small or medium-sized, in 6 large, but in only one case adherent to the brain stem. The test for optokinetic nystagmus is a simple procedure which is of importance in assessing the extent of the tumour and its relation to the brain stem, and it ought to be used as a routine in the oto-neurological diagnosis of acoustic neuromas.

Thanks to modern acoustic-vestibular and radiological diagnosis (Johnson & House 1964 Johnson, 1969; Pulec et al. 1964 Valvassori, 1969) it is now possible to detect and remove small neuromas. In patients having large tumours, with extinguished acoustic and vestibular function, it is of the utmost importance to be able to establish preoperatively the extent of the tumour and especially its relation to the brain stem. This applies especially to oto-neurosurgery in which the size of the tumour influences the choice of approach and method of operation. Monographs by House and his associates (1964 and 1968) have clearly shown that any sign from the cranial nerves, cerebellum, and brain stem is of great importance in assessing the extent of the tumour and its relation to the surroundings. In this paper we would like to draw attention to the role of optokinetic nystagmus which does not appear to have been utilized routinely in the diagnosis of acoustic neuroma.

Optokinetic nystagmus is a psychocortical reflex in which the eye follows the movements of stripes on a drum (Cords, 1926). Its afferent paths are afferent optic paths coursing from the eyes to the optic cortical centre in the area striata. The area striata is surrounded by an optomotor zone corresponding to Broman's zone 18 which houses the optomotor cortical centre. This centre receives fibres from the homolateral cortical visual field and, via the posterior part of the splenium corporis callosi, from the contralateral visual and optomotor field. The efferent optomotor paths assemble in the parietal lobe, beneath the supramarginal and angular gyri, course on the inside of Gratiolet's optic radiation, through the posterior part of the internal capsule and outer part of the cerebral peduncle to the mesencephalon, where they cross in the middle, ending in the contralateral gaze centre in the vicinity of the abducens nucleus. Lesions of the efferent path in the cerebellum will cause absent or defective horizontal optokinetic nystagmus to the contralateral side, lesions of the brain stem to the homolateral side. Several authors have confirmed Cords' hypothesis (Fox & Holmes, 1926 Kestenbaum, 1932, Cogan & Loeb 1949; Carmichael et al., 1954 Smith & Cogan 1959 Silverstein & Wiener 1965 Tos et al., 1972), finding abnormal optokinetic

nystagmus to the contralateral side in lesions of the posterior part of the hemisphere, mainly in the temporoparietal region.

Little has been published about abnormal horizontal optokinetic nystagmus in brain stem lesions. In lesions of the pons and cerebellum Fox & Holmes (1926) found two cases of unilateral abnormal optokinetic nystagmus to the homolateral side and one of bilaterally abnormal optokinetic nystagmus. We (Tos et al. 1972) have described 40 cases of vascular degenerative brain stem lesions and tumours of the brain stem and cerebellopontine angle in which the horizontal optokinetic nystagmus was abnormal to the side of the lesion in 37 cases and to the contralateral side in 3 Among another 40 patients with bilaterally abnormal horizontal optokinetic nystagmus the great majority had brain stem lesions.

Abnormal optokinetic nystagmus to the same side as acoustic neuromas, due to pressure upon the brain stem, has been reported by Enoksson (1956) in four patients. Simon & Gay (1964) have also described two cases of abnormal optokinetic nystagmus to the side of acoustic neuroma.

METHOD MATERIAL AND RESULT

In a total of 6 377 acoustic vestibular tests done during the period 1966–1970 investigation for horizontal and vertical optokinetic nystagmus was performed using a manually operated drum with black and white stripes at a distance of 40–50 cm from the patient's eyes. In all doubtful cases and in cases with defective or absent optokinetic nystagmus the investigation was done also by an electric drum having a constant angular velocity of 72, 180, and 360 °/sec respectively. Abnormal horizontal optokinetic nystagmus was found in 142 cases (14%) of diseases due to an organic, cerebral lesion, or in 2.2% of all tests for optokinetic nystagmus (Tos et al. 1972).

This material comprises 17 patients with operatively confirmed acoustic neuromas, as a rule large. The acoustic vestibular tests as well as the tests for optokinetic nystagmus were carried out by us, whereas the operations were performed by the neurosurgeons using the occipital approach and a neurosurgical technique. In 6 patients abnormal horizontal optokinetic nystagmus was found, in 4 of them absent to the same side in one defective to the same side as the tumour and in one absent to the opposite side. In 11 patients the optokinetic nystagmus was normal. The two groups of patients were analysed with a view to acoustic vestibular signs and signs from other cranial nerves as well as operative findings, with emphasis on the size of the tumour and especially its relation to the brain stem.

Comparison of the common signs in the two groups of patients (Table I) showed that anacusis, peripheral facial palsy reduced corneal sensibility and choked disc were relatively more frequent in the group with abnormal optokinetic nystagmus. However extinguished vestibular function and cerebellar signs, such as ataxia and dysdiadochokinesia, were more frequent in the group having normal optokinetic nystagmus.

X-ray examination of the internal pori acustici (Table II) showed normal appearances in 2 patients of the group with abnormal optokinetic nystagmus. In both, air encephalography revealed a large acoustic neuroma, and at operation a large tumour was found medially in the cerebello-pontine angle, compressing the brain stem and adhering firmly to it. Both patients had extinguished acoustic-vestibular function. In the group with normal optokinetic nystagmus the porus was normal in one patient whose tumour was large and extended towards the tentorium cerebri. In the remaining patients of both groups the porus acusticus internus was destroyed. Its width was increased in only 2 cases, one with a small and the other one with a medium-sized tumour In 9 cases Pantopaque cisternography or air encephalography was done,

Table I. *Signs in acoustic neuromas in relation to optokinetic nystagmus*

	No. of pats.	Hearing			Vestibular function		Facial palsy	Reduced corneal sensibility	Choked disc	Cerebellar signs
		Anacusis	Greatly impaired	Mildly impaired	Extinguished	Greatly impaired				
Abnormal Optokinetic nystagmus	6	4	1	1	4	2	2	3	3	2
Normal Optokinetic nystagmus	11	5	6	0	10	1	2	3	2	6

showing in all cases a tumour size consistent with the operative findings.

House's (1964) classification into small, medium-sized, and large tumours was also used. The small tumours, restricted to the internal auditory canal, and the medium-sized ones, extending into the cerebello-pontine angle without compressing the brain stem or the cerebellum, were found only in the group with normal optokinetic nystagmus (Table II). House (1964) calls large acoustic neuromas those which are large enough to compress the brain stem and cerebellum. All tumours from the group with abnormal optokinetic nystagmus and almost half the tumours from the group with normal optokinetic nystagmus were large. In the operative reports some of the large tumours were described as being very large, the majority belonging to the group with abnormal optokinetic nystagmus.

The relation of the tumour to the brain stem and its adhesion to this structure was accurately described in all cases but one. In the group with abnormal optokinetic nystagmus 5 patients had tumours which adhered firmly to the brain stem (Table II) and caused great difficulties in detachment. In four the capsule was so firmly fixed to the brain stem that it had to be left behind. In one case of this group it was not possible to glean from the description whether or not the tumour had been fixed to the brain stem, but it was very large, weighing 50 g. Within the group having normal optokinetic nystagmus only one patient had a tumour adhering to the brain stem. It was only partially removed.

Table II. *X-ray findings concerning the internal auditory canal and operative findings in relation to optokinetic nystagmus*

	X-ray findings Internal auditory canal		Operative findings					
			Size of tumour					
	Normal	Destroyed	Small	Medium	Large	Very large	Adhering to brain stem	Partial removal
Abnormal Optokinetic nystagmus	2	4	0	0	2	4	5	4
Normal Optokinetic nystagmus	1	10	1	4	5	1	1	1

In all the other patients of this group the tumour could be demarcated from the brain stem with relative ease and removed in toto.

DISCUSSION

Abnormal horizontal nystagmus is no doubt due to considerable pressure upon the brain stem. Half the patients showing this abnormality also had choked disc (Table I), and all the tumours were large most of them very large (Table II). The optomotor paths end in the pontine gaze centre in the vicinity of the abducens nucleus (Cords, 1926) which is situated in the very area which is most often in contact with the tumour Destruction of the gaze centre ought to entail horizontal gaze paralysis, but this was not found in the cases with acoustic neuromas. In other brain stem lesions we have often found gaze paralysis co-existing with unilaterally or bilaterally abnormal horizontal optokinetic nystagmus (Tos et al. 1972) In acoustic tumours the gaze centre is affected by pressure, but probably not destroyed. Possibly less pressure is required to cause abnormal optokinetic nystagmus than horizontal gaze paralysis, and possibly the pontine centre for optokinetic nys- is in a given—laterally situated—area the gaze centre and is therefore affected the remaining parts of the centre.

True Pantopaque cisternography and all other findings afford important information concerning the size of the tumour and its relation to the surroundings, but to the surgeon any information about the relation of the tumour to the brain stem must be of great importance. House (1964) has accurately described the problems and risks concerning the brain stem in removing large tumours and that part of the capsule which adheres to the brain stem However these problems in removing the capsule are not encountered in all large tumours, and it is therefore important to be able to establish preoperatively in which patients such problems are to be anticipated. In all patients with abnormal optokinetic nystagmus, difficulties were encountered owing to the close relation of the tumour to the brain stem in removing the capsule which often had to be left behind. This is in contradistinction to the group having normal optokinetic nystagmus in which such difficulties were considerably less marked and in which the capsule could be removed in toto in all cases but one. Accordingly the test for optokinetic nystagmus seems to make up an essential supplement to cisternography and other diagnostic procedures. It may contribute to the choice of approach and to the preoperative decision as to whether the tumour can be removed totally or partially And after the operation it gives a hint as to whether the pressure upon the brain stem has been relieved.

In the present analysis the patients were grouped by horizontal optokinetic nystagmus. In 4 with abnormal horizontal optokinetic nystagmus there were also signs of abnormal vertical optokinetic nystagmus, downwards, upwards, or into both directions. In all patients with normal horizontal optokinetic nystagmus the vertical optokinetic nystagmus was normal too. Vertical optokinetic nystagmus is generally not used in otoneurological diagnosis as the course of its paths is still not sufficiently known. Among 6 377 tests for optokinetic nystagmus we (Rosborg et al., 1972) found 97 patients to have abnormal vertical and normal horizontal nystagmus. Since the majority exhibited signs of organic disease of the central nervous system we believe that vertical optokinetic nystagmus is of a certain value in otoneurological diagnosis. Among 124 patients with organic diseases of the central nervous system and abnormal horizontal optokinetic nystagmus we (Tos et al., 1973) found 95 to have also abnormal vertical optokinetic nystagmus, indicating that the paths for vertical optokinetic nystagmus course along those for horizontal optokinetic nystagmus. They end in the vertical gaze centre in the mesencephalon, situated in the interstitial nucleus (Cajal) on a level

with the oculomotor nuclei (Szentágothai, 1943), i.e. only a bit higher than the pontine centre for horizontal optokinetic nystagmus. Therefore, the occurrence of abnormal vertical optokinetic nystagmus in acoustic neuromas is not surprising.

In our opinion systematic tests for horizontal and vertical optokinetic nystagmus, possibly by electronystagmographic recording, should be included as a routine method in the diagnosis of acoustic neuromas. In the large centres where many operations are done for acoustic neuromas—which are delimited tumours exerting primary pressure upon a delimited area—such tests will be able to throw further light upon the pathophysiology of optokinetic nystagmus.

ZUSAMMENFASSUNG

Bei 17 Patienten mit operativ bewiesenen, meistens grossen Akustikusneurinomen wurde optokinetischer Nystagmus in bezug auf Grösse und Verbindung des Neurinoms zum Gehirnstamm analysiert. Bei sechs Patienten war der optokinetische Nystagmus abnormal; bei diesen war der Tumor gross oder sehr gross und in den meisten Fällen zum Gehirnstamm adhärent. Abnormal optokinetischer Nystagmus wird durch Druck des Tumores auf das pontine, optokinetische Zentrum verursacht. Bei 11 Patienten war der optokinetische Nystagmus normal, bei 5 von diesen war der Tumor klein oder mittelgross und bei 6 gross, aber nur bei einem adhärent zum Gehirnstamm. Die optokinetische Probe ist einfach, aber bedeutungsvoll, um die Grösse und die Beziehung des Tumors zum Gehirnstamm festzustellen. Sie sollte eine Routinemethode bei der oto-neurologischen Diagnostik der Akustikustumoren sein.

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Acta Otolaryng 76 244–253 1973

DISTRIBUTION AND ORIGIN OF ADRENERGIC NERVE FIBERS IN THE VESTIBULAR APPARATUS AND THEIR ARTERIAL SUPPLY IN THE GUINEA PIG

A Fluorescent Microscopic Study

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(Received December 12, 1972)

Abstract Fine adrenergic nerve fibers which are distributed to the saccular and utricular maculae, ampullar capsules and ducts of the semicircular canals and vestibular nerve of the guinea pig were observed by fluorescent microscopy These fibers were divided into three groups according to their course and location perivascular nerve trunk, and independent groups. The arteries leading to the vestibular organs contained many adrenergic fibers. The uni- and bilateral cervical sympathetic denervations at various levels showed that the adrenergic fibers in the vestibular organs originated in the ipsilateral superior cervical ganglion. Regarding the arteries leading to the vestibular organs, the basilar anterior inferior cerebellar labyrinthine and common cochlear arteries receive adrenergic fibers from the bilateral superior cervical ganglia, while the peripheral branches of the labyrinthine artery receive their adrenergic fibers only from the ipsilateral superior cervical ganglion. These adrenergic fibers come from the ganglion via the internal carotid nerve

Disturbance of sympathetic innervation of the vestibular apparatus has been assumed to be one of the causes of vestibular diseases such as vertigo ataxia and Menière s disease. Therefore effort has been made to examine the autonomic nerve fibers in the vestibular organs (for extensive reviews, see Lindeman 1969). However the distribution and origin of the sympathetic nerves in the vestibular organs still remain almost obscure because sympathetic nerve fibers cannot be stained selectively by means of conventional histological methods for nerve fibers. Since postganglionic sympathetic fibers contain noradrenalin as a transmitter substance, they are called adrenergic fibers. At present, the fluorescent histochemical method of Falck and Hillarp (Falck et al. 1962 Falck, 1962, Falck & Owman 1965) by which noradrenalin is converted into green-yellowish flurescent substance within the tissues, is accepted as the most reliable and specific method for visualization of adrenergic fibers. So far no fluorescent microscopic study on adrenergic fibers in the vestibular apparatus has been reported except for a brief description by Spoendlin & Lichtensteiger (1966) They found adrenergic fibers in the utricular macula and crista of the semicircular canal.

In general, it is known that the specific fluorescence of adrenergic fibers disappears after sectioning of the postganglionic fibers or after extirpation of the ganglion in which they originate, whereas it does not disappear after sectioning of the preganglionic fibers (Falck 1962 Malmfors, 1965 Terayama et al. 1966 Spoendlin & Lichtensteiger 1967). Thus, it is possible to ascertain which ganglion or nerve is the origin of adrenergic fibers in the organ.

Using the method of Falck and Hillarp, the

present authors attempted to chart the distribution of adrenergic fibers in the vestibular organs of the guinea pig and to ascertain the origin of the adrenergic fibers by section of the cervical sympathetic chain at various levels.

Furthermore, since disturbance of the autonomic nervous system of the blood vessels distributing to the vestibular organs is thought to induce vestibular symptoms (Beickert, 1962), the adrenergic innervation to the blood vessels at various levels from the basilar artery to the branches of the labyrinthine artery was also examined.

MATERIAL AND METHOD

Sixty-one guinea pigs weighing between 150 and 300 g were used. After decapitation, the temporal bone was removed, and the vestibular space and the internal auditory meatus were opened. After removal of the cochlea and cochlear nerve trunk, the osseous canals of the saccular and utricular nerves were opened. The saccular and utricular maculae, the ampullae and ducts of the semicircular canals, branches of the vestibular nerve and the arteries running with these nerves were immediately removed and mounted on microscopic slide glass without fixation or decalcification. The basilar artery and its branches distributing to the vestibular organs were cut into small pieces under a binocular microscope and mounted on slide glass. The slide glass was dried within a desiccator containing silica gel and then exposed to formaldehyde gas in a closed vessel at 80 C for an hour. The specimens on the slide glass were immersed with Entellan (Merck) and covered with a coverglass. They were observed under an Olympus fluorescent and phase contrast microscope through a dark-field condensor. The exciting light was provided by a high pressure mercury lamp of 200 W and light of 510 nm in wavelength was absorbed through filters. For control material, the iris was mounted on the same slide. In order to counterstain the blood vessels, several guinea



Fig. 1. Lower border of the macula sacculi of the normal guinea pig. Adrenergic fibers (*arrows*) course parallel to the saccular nerve fibers. *O*: otolith. × 110.

pigs received a single intracardiac injection with 0.5 ml of 2% Evans Blue solution before sacrifice, because Evans Blue represents a red fluorescence and the blood vessels are revealed in good contrast to green-yellow fluorescence of adrenergic fibers (Udenfriend, 1962, Hamberger & Hamberger 1966).

In order to ascertain the origin of adrenergic fibers in the vestibular organs and arteries as described above, sectioning of the cervical sympathetic trunk inferior to the superior cervical ganglion, extirpation of the superior cervical ganglion and section of the internal carotid nerve which is the largest branch of the ganglion were carried out uni- and bi-laterally. The animals were sacrificed 3–26 days after sympathectomy. For each sympathectomy 5 animals were used.

Reserpine was injected intraperitoneally 10 mg/kg in 3 animals 24 hours before sacrifice in order to examine whether the specific fluorescence detected was due to noradrenalin (Fuxe & Sedvall, 1964, Malmfors, 1965).

FINDINGS

Adrenergic fibers in the vestibular apparatus were recognized as fine varicose green-yellow or green fluorescent fibers. Their fluorescence



Fig. 2. (*a*) Medial area of the macula utriculi of the unoperated side 3 days after extirpation of the unilateral superior cervical ganglion of the opposite side. Many varicose fluorescent fibers enter the macula together with the utricular nerve fibers. Fluorescent fibers are not influenced by the operation: *B*, bone chips. × 300. (*b*) Schematic drawing of the areas corresponding to Figs. 2 and 3 in the macula utriculi.

disappeared 24 hours after injection of reserpine. The blood vessels stained with Evans Blue showed red fluorescence

It was necessary to remove the otolith membrane from the maculae otherwise, the specific fluorescence would have been obscure. In the saccular macula, adrenergic fibers were distributed sparsely They appeared to run parallel to the saccular nerve fibers (Fig. 1)

The adrenergic fibers, accompanied by the saccular and utricular nerve fibers, entered the saccular and utricular macula (Figs. 2*a* and 7), and extended to the border of the macula beyond the sensory epithelial area (Fig. 3*a*). Most of the adrenergic fibers evidenced no relation with the blood vessels, but a few of them ran along the blood vessels (Fig. 3*b*). The membrane of the utricular duct showed

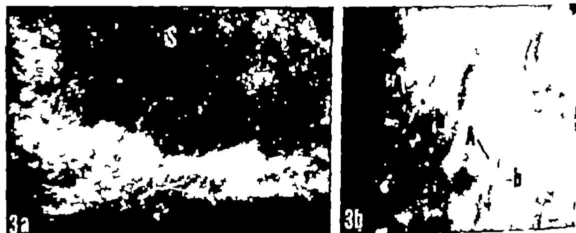


Fig. 3. (*a*) Border of the macula utriculi of the unoperated side 4 days after extirpation of the unilateral superior cervical ganglion. Fluorescent fibers appear to exceed the sensory cell area (*S*). 150. (*b*) Central area of the macula utriculi from an untreated animal. Some of the adrenergic fibers (*A*) course along the blood vessels (*b*) 230.



Fig. 4 The lateral semicircular canal with the ampullar nerve (*N*) demonstrates that the adrenergic fibers accompanied by ampullar nerve fibers enter the crista (*C*) and ampulla. The ampullar capsule was not opened. Eight days after the unilateral superior cervical ganglionectomy Unoperated side: *B* bone chip. 55

no specific fluorescence. The relationship between the adrenergic fibers and the sensory cells in the maculae were obscure for technical reasons. Also it was not possible to ascertain in which layer the adrenergic fibers coursed.

In the three semicircular canals, adrenergic fibers showed similar distribution patterns. As illustrated in Fig. 4 the adrenergic fibers were seen to enter the crista ampullaris together with the ampullar nerve fibers. Then they were scattered to the ampullar capsule and canal portion. These fibers appeared to leave from several points on the sloping portion of the crista (Fig. 5 *a*). Several adrenergic fibers were found close to the perilymphatic surface of the endolymphatic duct of the semicircular canal (Fig 6) Some of the fibers in the capsule and canal portion coursed close to the blood vessels (Figs. 5 *a* and 6 *b*)

Very fine adrenergic fibers were detected within all the branches of the vestibular nerve distributing to the respective vestibular organs

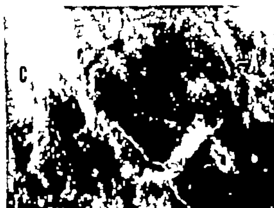




Fig. 5 (*a*) The anterior semicircular canal of the normal animal. The ampullar capsule was opened. The adrenergic fibers appear to start from points (*arrows*) on the sloping portion of the crista (*C*) to distribute in the ampullar capsule and canal portion. An adrenergic fiber (*A*) courses along the blood vessel. 160. (*b*) The area corresponding to (*a*) in the ampulla of the lateral semicircular canal. Operated side 4 days after section of the unilateral internal carotid nerve. No fluorescent fibers are observed: *C* the crista. 300

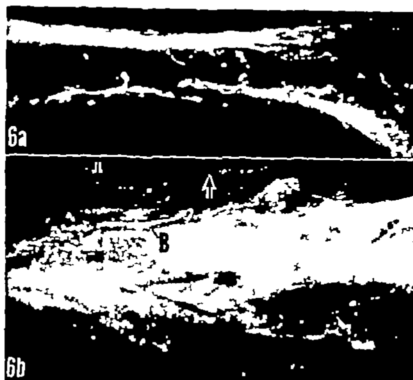


Fig. 6. (*a*) Adrenergic fibers surrounding the canal portion of the anterior semicircular canal in the normal animal. The ampulla to right. × 150. (*b*) The canal portion of the lateral semicircular canal of the unoperated side 4 days after section of the unilateral internal carotid nerve. Note adrenergic fibers travel along the blood vessels (*B*). × 200.

(Figs. 4 and 7). Most of them appeared to run parallel to the vestibular nerve fibers without any connection with the blood vessels. They were more numerous within the nerve trunk than in the peripheral vestibular organs innervated.

A comparison of the adrenergic fibers in the vestibular organs to those in the iris (Fig. 8) revealed that the fibers in the former were smaller in diameter and less numerous in number than in the latter.

The arteries running together with the vestibular nerve trunk in the osseous canals had networks of adrenergic fibers (Fig. 9). It could not be ascertained, however, whether or not the perivascular adrenergic network supplied

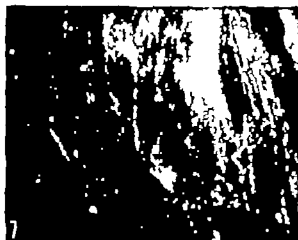


Fig. 7. The distal stump of the saccular nerve of the normal guinea pig. Fine adrenergic fibers run among the saccular nerve fibers. × 290.

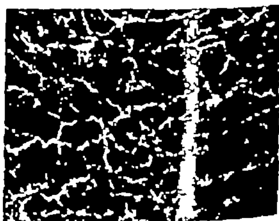


Fig. 8. The adrenergic fibers in the iris of the normal guinea pig. They are more numerous in number and larger in diameter than those in the vestibular apparatus. × 135.



Fig. 9 ( ) The anterior vestibular artery of the normal guinea pig. 250. (*b–c*) The vestibulo-cochlear arteries of both sides of the same animal 4 days after extirpation of the unilateral superior cervical ganglion. The adrenergic fibers on the unoperated side (*b*) are unchanged but totally absent on the operated side (*c*). (*b*), 200. (*c*), 250.

adrenergic fibers to the vestibular nerve and the peripheral organs.

The wall of the basilar artery and its branches had dense networks of adrenergic fibers (Figs. 9–12). The adrenergic fibers of the networks were more intensely fluorescent and larger in diameter than the adrenergic fibers in the vestibular organs. The perivascular adrenergic fibers gradually decreased in number as they ran toward the periphery

Unilateral sympathectomy

Sectioning of the unilateral cervical sympathetic trunk inferior to the superior cervical ganglion did not influence the specific fluorescence of the adrenergic fibers in either the vestibular apparatus or the arteries on both sides.

Findings obtained from the unilateral sectioning of the internal carotid nerve were identical with those findings obtained after the unilateral superior cervical ganglionectomy

After the unilateral extirpation of the superior cervical ganglion the observations of the adrenergic fibers in the peripheral vestibular areas were different from those of the adrenergic fibers in the arteries central to the vestibular organs. The adrenergic fibers in the peripheral vestibular organs, the branches of the vestibular nerve and the peripheral divisions of the labyrinthine artery disappeared completely on the operated side (Figs. 5 *b* and 9 *c*) but remained unchanged on the unoperated side (Figs. 2 *a*, 3 *a*, 4 6 *b* and 9 *b*). The adrenergic fibers surrounding the anterior inferior cerebellar labyrinthine and common cochlear arteries remained even on the operated side but were prominently reduced in number (Figs. 11 *b* and 12 *b*) Those remaining fibers could be followed as far as the entrance of the internal auditory meatus. The adrenergic fibers on the unoperated side appeared unchanged (Figs. 11 *c* and 12 *a*). The fluorescent fibers of the basilar artery were also unchanged in appearance (Fig. 10 *a*).

Bilateral sympathectomy

Removal of the bilateral superior cervical ganglia and section of the bilateral internal carotid nerves resulted in the complete disappearance of the specific fluorescent fibers in both the vestibular organs and in the arteries from the basilar artery to the peripheral branches of the labyrinthine artery on both sides (Figs. 10 *b* 11 *d* 11 *e* and 12 *c*).

DISCUSSION

Using fluorescent histochemical method of Falck and Hillarp the authors observed specific fluorescent fibers in the vestibular organs

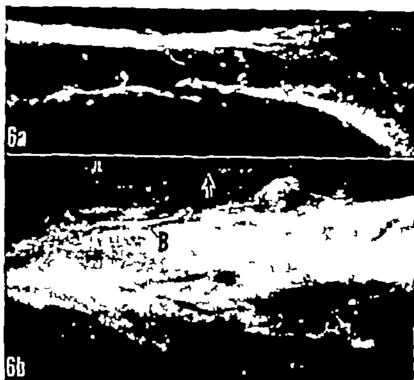


Fig. 6 (*a*) Adrenergic fibers surrounding the canal portion of the anterior semicircular canal in the normal animal. The ampulla to right. × 150. (*b*) The canal portion of the lateral semicircular canal of the unoperated side 4 days after section of the unilateral internal carotid nerve Note adrenergic fibers travel along the blood vessels (*B*) 200.

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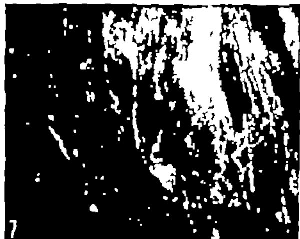


Fig. 7 The distal stump of the saccular nerve of the normal guinea pig. Fine adrenergic fibers run among the saccular nerve fibers. 290.

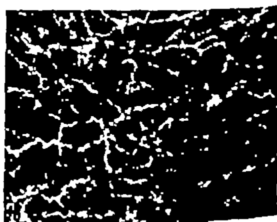


Fig. 8 The adrenergic fibers in the iris of the normal guinea pig. They are more numerous in number and larger in diameter than those in the vestibular apparatus. × 135



Fig. 12. The labyrinthine artery (*a*) Unoperated side 3 days after the unilateral superior cervical ganglionectomy Adrenergic fibers are unchanged in appearance. 120. (*b*) Operated side 4 days after the unilateral superior cervical ganglionectomy Adrenergic fibers still remain but markedly reduced in number 190. (*c*) Operated side 3 days after sectioning of the bilateral internal carotid nerves. No adrenergic fiber found. 255

al (1966) and Ross (1971). The adrenergic fibers in the maculae and ampullar cristae appeared to enter in association with the vestibular nerve fibers. According to Spoendlin & Lichtensteiger (1966) adrenergic fibers independent of the blood vessels in the cochlea disappeared after transsection of the cochlear nerve in the internal auditory meatus. This would also be the case with the supply of adrenergic fibers from the vestibular nerve to the macula and crista.

The adrenergic fibers contained in the vestibular nerve present fine and distinct fluorescence Such a feature is characteristic of the preterminal and terminal portions of adrenergic fibers (Falck, 1962, Malmfors, 1965 Malmfors & Sachs, 1965) The adrenergic fibers contained in the vestibular nerve are more numerous than those in the macula and ampulla. Adrenergic fibers, therefore, may be in some functional relationship with the vestibular nerve. Spoendlin & Lichtensteiger (1966) proposed that adrenergic fibers in the cochlea may influence the threshold of the cochlear neurons. It is of great interest if it is also true for the vestibular neurons.

3 *Independent group* This group consists of adrenergic fibers related neither to blood vessels nor to vestibular nerve fibers. Such fibers were observed in the border of the macula, the capsula of the ampulla and the duct of the semicircular canal. These areas have been considered lacking in nerve fibers. Spoendlin & Lichtensteiger (1966) denied the presence of adrenergic fibers in the duct of the semicircular canal. The adrenergic fibers in the duct portion appeared to exist in the perilymphatic space close to the endolymphatic duct. This space contains fibrocytes, collagen fibrils and capillary blood vessels under the epithelial layer of the endolymphatic duct according to the authors electronmicroscopic observation. Since noradrenalin released from the adrenergic fibers may be diffusible in the perilymph, the adrenergic fibers may serve functionally as vasomotor and/or regulator of secretion or absorption of endo- or perilymph.

Furthermore, since the adrenergic fibers in the vestibular organs are far fewer in number and smaller in diameter than those in the iris, the direct adrenergic control of the vestibular organs would be relatively weak, as pointed out by Suga & Snow (1969) in a pharmacological study of adrenergic control of the cochlea.

As another adrenergic control to the vestibular organs, there is an abundance of adrenergic fibers distributing to the arteries related to these organs. Spoendlin & Lichtensteiger (1967), Nielsen & Owman (1967), Ohgushi (1968) and Kajikawa (1969) previously recognized the presence of dense networks of adrenergic fibers in the vertebrobasilar artery and its branches at the base of the brain in the cat, dog, rabbit and rat by means of fluorescent method of Falck and Hillarp. These researchers, with the exception of Spoendlin et al., made uni- as well as bilateral sympathetic denervation, and concluded that the adrenergic fibers of the main arteries of the brain originated exclusively in the superior

cervical ganglion and that those of the basilar artery were derived from the bilateral ganglia.

Regarding the origin of the adrenergic fibers, the authors findings obtained from the use of sympathectomized animals showed that the origins of the adrenergic fibers in the vestibular organs and in their related arteries did not exist below the level of the superior cervical ganglion because cervical sympathectomy below the level of this ganglion did not influence the specific fluorescence in these organs and in the arteries on both sides.

After unilateral superior cervical ganglionectomy the adrenergic fibers in the vestibular organs and in the peripheral branches of the labyrinthine artery disappeared totally on the operated side, but the adrenergic fibers remained unchanged on the unoperated side. These findings suggest that the adrenergic fibers originate exclusively in the ipsilateral superior cervical ganglion. Similar results have already been obtained in previous studies on the origin of adrenergic fibers in the cochlea which is embryologically analogous to the vestibular organs (Terayama et al., 1966 Spoendlin & Lichtensteiger 1967).

The arteries coursing centrally to the entrance of the internal auditory meatus on the operated side still showed the presence of adrenergic fibers, although prominently decreased in number after unilateral superior cervical ganglionectomy The corresponding arteries on the contralateral side appeared unchanged. However since the adrenergic fibers in the vestibular organs and their related arteries completely disappeared on both sides after bilateral superior cervical ganglionectomies or sectioning of the bilateral internal carotid nerves, it is reasonable to assume that the adrenergic fibers surviving on the operated side following unilateral ganglionectomy are derived from the contralateral superior cervical ganglion. In other words, the basilar artery anterior inferior cerebellar artery labyrinthine artery and common cochlear artery receive their adrenergic fibers from the bilateral superior cervical ganglia. Since these findings after ganglionectomy were the same as those obtained following section of the internal carotid nerve, the adrenergic fibers in the vestibular organs and arteries come via the internal carotid nerve.

Spoendlin & Lichtensteiger (1967) and Terayama (1970) made respectively fluorescent microscopic and electronmicroscopic studies on the origin of adrenergic fibers in the cochlea on the animals following unilateral superior cervical ganglionectomy and they surmised that the adrenergic fibers, which remained in the arteries proximal to the labyrinthine or common cochlear artery on the operated side, originated in the lower cervical ganglion, probably the stellate ganglion. But as seen from the present findings, this is not true.

This present study revealed the general adrenergic innervation of the vestibular organs; further studies will be necessary to clarify the relationship between adrenergic fibers and the cell components of the organs.

ACKNOWLEDGMENT

The authors wish to express their thanks to Dr In, Professor of Department of Anatomy and Dr Hirano Professor of Department of Otolaryngology Hokkaido University for their kind criticism, and to Dr C. Tanaka Assistant Professor of Department of Pharmacology Kyoto University for giving us valuable advice.

ZUSAMMENFASSUNG

Adrenergische Nervenfasern in der Macula sacculi und utriculi, der Kapsel der Ampulla und dem Kanal des Bogengangs und in Vestibularisnerven des Meerschweinchens wurden fluorescent mikroskopisch beobachtet. Diese Fasern wurden nach Verlauf und Lokalisation in drei Gruppen eingeteilt: die perivaskuläre, Nervenstamm- und independente Gruppe. Die die vestibulären Organe versorgenden Arterien sind auch reich an adrenergischen Fasern. Die uni- und bilateralen zervikalen sympathischen Denervationen in verschiedener Höhe ergaben, dass die adrenergischen Fasern im Vestibularapparat vom ipsilateralen Ganglion cervicale superior angeben. Die Arterien, die den Vestibularapparat versorgen (A. basilaris, A. cerebelli inferior anterior, A. labyrinthi und A. cochlea communis), werden durch die adrenergischen Fasern aus den bilateralen Ganglion cervicale superior innerviert, während die peripheren Äste der A. labyrinthi nur

durch die adrenergischen Fasern aus dem ipsilateralen Ganglion cervicale superior innerviert werden. Diese adrenergischen Fasern stammen aus dem Ganglion über N. caroticus internus.

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Acta Otolaryng 76 254–267 1973

THE CAPILLARY AREAS IN THE RABBIT COCHLEA

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(Received January 10 1973)

Abstract. The vascular system of the rabbit cochlea was demonstrated by the aid of contrast injection. The capillary areas are principally very similar to the human and guinea pig cochlea. The main differences are the existence of sparse capillaries on the vestibular membrane and the absence of the vessel of the basilar membrane. The vessel of the spiral prominence appears as the most important capillary vessel of the external wall, according to its rich vascular supply. The absence of the vessel under the tunnel of Corti makes the rabbit particularly interesting for hearing research concerning the complicated question of the oxygen supply to the organ of Corti.

In spite of the fact that the rabbit has been much used in cochlear research very few investigations are available which study the ~ular anatomy of its cochlea. In a detailed ological description of the rabbit coch- Retzius (1884) only briefly mentions some cochlear vessels. Retzius observed capillaries on the vestibular membrane. The vessel of the basilar membrane (vas spirale) was demonstrated in the rabbit embryo but later atrophied to a fibrous strand. The irregular running limbus vessels were also observed by Retzius.

Nabeya (1923) demonstrated the similarity of the rabbit and the guinea pig cochlea and noted that the vascular pattern was less complex in the former. Nabeya also found an interesting difference of the rabbit in generally having only one spiral vessel in the spiral lamina. However a vessel was sometimes demonstrated under the tunnel of Corti. Nabeya also found some capillary vessels in the rabbit vestibular membrane contrary to the guinea pig. Nabeya's description was accompanied by some semi-schematic drawings.

The morphological structure of the spiral prominence in the rabbit has been investigated by Borghesan (1969).

In certain animals with hereditary deafness, the vessel of the basilar membrane (vas spirale) has been shown to degenerate or to be entirely absent, e.g. the Hedlund white mink (Hilding et al., 1967) the shaker I mouse (Kikuchi & Hilding, 1967) and the deaf Dalmatian dog (Andersson et al. 1968). Contrarily the systematic investigations of all cochlear vessels in the deaf waltzing guinea pig did not reveal any abnormalities of the vascular pattern (Axelsson & Ernstson, 1972). The degeneration or absence of the vessels close to the organ of Corti has been suggested to be responsible for the hearing defect. The finding has also been much used in the discussion concerning the oxygen supply to the organ of Corti. However in most papers concerned with the function and significance of the cochlear vessels only the stria vascularis and the vessel under the organ of Corti are examined and other cochlear vessels omitted.

From the sparse information available on the vascular pattern of different mammals it

This work was supported by the Swedish Research Council project B 72-12X 2835-03 and by the Swedish Board for Technical Development project 72 1500 U 1170.



Fig. 1 Modiolus of the rabbit cochlea. The spiral modiolar artery (*SMA*) runs a serpentine course around the modiolus. The spiral modiolar vein (*SMV*) runs more straight. At the base the spiral modiolar vein and the vein of the round window (*VRW*) form the vein of the cochlear aqueduct (*VCAQ*).

appears that most mammals have two spirally running vascular arcades in the spiral lamina, one peripheral under the tunnel of Corti and one central in the tympanic lip (for references see Axelsson, 1968) However in the cat (Smith, 1954) in the mink (Hilding et al. 1967) and in the rabbit (Retzius, 1884 Nabeya, 1923) only one marginal spiral vessel has been demonstrated. Since the vessel of the basilar membrane (vas spirale) is situated closest to the hair cells, it has also been held responsible for the oxygen supply to the sensory organ. When the vessel of the basilar membrane is absent, the oxygen supply apparently must derive from other sources.

The aim of the present investigation was to study the vascular anatomy of the rabbit cochlea towards the above questions.

MATERIAL AND METHOD

The material comprises 14 pairs of rabbit cochlea, of which five were young, about 3 weeks of age and nine were adult of various ages. All exhibited a normal Preyer s reflex. The demonstration of the cochlear blood vessels was achieved by a previously described method (Axelsson, 1968, 1971 1972) which in summary contains the following preparative steps:

Anesthesia with intravenous pentobarbital solution.
Perfusion of the vascular system with Ringer *solution.*
Injection of a contrast, Prussian blue solution in the vascular system.
Fixation of the cochlea.
Decalcification.



Fig. 2 Radiating arterioles (*RAL*) leaving the spiral modiolar artery (*SMA*) have a pronounced serpentine course but do not form spring-coil appearing structures, „glomeruli" as in the guinea pig. *SMV* = spiral modiolar vein.

Acta Otolaryng 76 254–267 1973

THE CAPILLARY AREAS IN THE RABBIT COCHLEA

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(Received January 10, 1973)

Abstract The vascular system of the rabbit cochlea was demonstrated by the aid of contrast injection. The capillary areas are principally very similar to the human and guinea pig cochlea. The main differences are the existence of sparse capillaries on the vestibular membrane and the absence of the vessel of the basilar membrane. The vessel of the spiral prominence appears as the most important capillary vessel of the external wall, according to its rich vascular supply. The absence of the vessel under the tunnel of Corti makes the rabbit particularly interesting for hearing research concerning the complicated question of the oxygen supply to the organ of Corti.

In spite of the fact that the rabbit has been much used in cochlear research, very few investigations are available which study the vascular anatomy of its cochlea. In a detailed morphological description of the rabbit cochlea Retzius (1884) only briefly mentions some cochlear vessels. Retzius observed capillaries on the vestibular membrane. The vessel of the basilar membrane (vas spirale) was demonstrated in the rabbit embryo but later atrophied to a fibrous strand. The irregular running limbus vessels were also observed by Retzius.

Nabeya (1923) demonstrated the similarity of the rabbit and the guinea pig cochlea and noted that the vascular pattern was less complex in the former. Nabeya also found an interesting difference of the rabbit in generally having only one spiral vessel in the spiral lamina. However a vessel was sometimes demonstrated under the tunnel of Corti. Nabeya also found some capillary vessels in the rabbit vestibular membrane contrary to the guinea pig. Nabeya's description was accompanied by some semi-schematic drawings.

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Fig. 4. Spiral lamina, basal turn. Radiating arterioles supply and collecting venules drain the vascular arcades in the tympanic lip forming a vascular margin. *In the most basal portion the vascular arcades do not* form a well-defined spiral vessel in the tympanic lip (right). More apically the vascular arcades form the vessel of the tympanic lip (*VSTL*) (left). There is no vessel under the outer hair cells (*OHC*) and no vessels crossing over the basilar membrane to the external wall. *IHC* = *inner hair cells. Arrow indicates attach*ment of the vestibular membrane.

more serpentine than in the human cochlea but which do not have the spring-coil appearance ("glomeruli") of the guinea pig (Fig. 2). The rabbit cochlea is drained by the *spiral modiolar vein* which also runs a spiral course around the modiolus from the apex to the base (Fig. 1). The vein is situated in the central angle of the scala tympani immediately below the spiral ganglion. The spiral modiolar vein receives many *collecting venules* on its convex and apical aspects. These collecting venules derive from all three scalas in the external wall, from the spiral lamina and limbus and from the capillary areas in the modiolus. The *spiral modiolar vein* is similar to the same vein in the guinea pig.

Capillary Areas

Modiolus

The capillary areas are situated in the *acoustic nerve* in the *spiral ganglion* and in the *modiolus wall*. As in the guinea pig and human cochlea these areas lack any definite regular vascular pattern. There is a fairly dense net of delicate capillaries running in different directions (Fig. 3).

Spiral lamina and limbus

The following vascular structures can be identified in the spiral lamina and limbus (Figs. 4–7):



Fig 5 Spiral lamina, basal turn, apical portion. The vascular arcades form a spiral vascular margin, the vessel of the tympanic lip (*VSTL*). There is no vessel under the outer hair cells (*OHC*). IHC – Inner hair cells.

diating arterioles
flecting venules
he vessel of the tympanic lip
The limbus vessels.

The radiating arterioles supplying the capillary vessels are branches of the radiating arterioles deriving from the spiral modiolar artery. They run centrifugally over the spiral lamina. Each radiating arteriole supplies a small segment. The branches ramify and terminate as vascular arcades which are drained by centripetally running *collecting venules*. In the basal turn, particularly in the most basal portion, these arcades do not form a well-defined spiral vascular margin as in the guinea pig and human cochlea but more open and curved vascular arcades in the tympanic lip (Fig. 4). In the apical parts of the basal turn and in the second and third turn there is a well-defined spiral marginal vessel in the tympanic lip with few interruptions of the marginal border the *vessel of the tympanic lip* (Fig. 5). In no cochlea could any vessel of the basilar membrane be demonstrated. The region of the spiral lamina peripheral to the vessel of the tympanic lip, including the whole basilar membrane, is consequently avascular. In no case could any vascular connection between the vessels of the spiral lamina and the external wall be demonstrated as appears in man. The capillaries of the spiral limbus form an uneven peripheral marginal vascular border in the spiral limbus, *the limbus vessels* similar to the guinea pig and human cochlea (Fig. 6). The vascular system

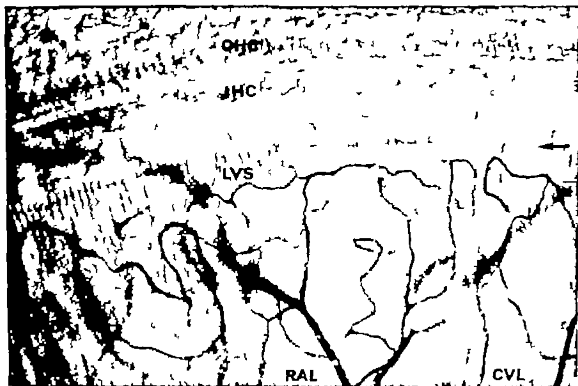


Fig. 6. Spiral limbus, basal turn. Radiating arterioles (*RAL*) supply and collecting venules (*CVL*) drain the uneven capillary vascular margin of the spiral limbus, the limbus vessels (*LVS*). *OHC* = outer hair cells. *IHC* = inner hair cells. Arrow indicates Huschke's dents, i.e. limbus cells.

in the most apical parts of the cochlea is more sparse and open but all the defined vascular structures can be demonstrated (Fig. 7).

Vestibular membrane

In the guinea pig and human cochlea the vestibular membrane is completely avascular. In the rabbit cochlea there are sparse delicate capillaries interconnected to the vessel at the vestibular membrane in the external wall and to the radiating arterioles in the spiral lamina (Fig. 8). The sparse capillaries of the vestibular membrane were also observed by Retzius (1884) and Nabeya (1923).

External Wall

Scala vestibuli

There are four well-defined vascular structures in the scala vestibuli:

Radiating arterioles
The vessel at the vestibular membrane
A capillary net above the vestibular membrane
Collecting venules.

The radiating arterioles from the spiral modiolar artery run a serpentine centrifugal course centrally in the scala vestibuli. More peripherally they straighten, ramify and supply all the capillary areas in the external wall (Fig. 9). Immediately above the attachment of the vestibular membrane there is a spirally running vessel, *the vessel at the vestibular membrane* which is supplied by the radiating arterioles and drained by *collecting venules* from the scala tympani (Fig. 10). The vessel at the vestibular membrane is more easily demonstrated in the basal turn and consists of short segments with frequent interruptions in



Fig 5 Spiral lamina, basal turn, apical portion. The vascular arcades form a spiral vascular margin, the vessel of the tympanic lip (*VSTL*). There is no vessel under the outer hair cells (*OHC*). IHC – Inner hair cells.

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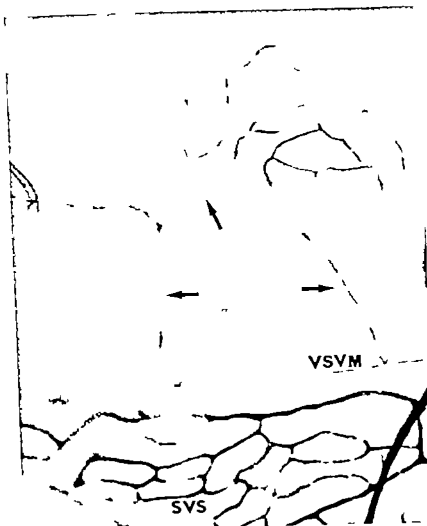


Fig. 8. The vestibular membrane with sparse capillaries (*arrows*). *VSVM* = the vessel at the vestibular membrane. *SVS* = stria vascularis.

tions consequently are the supplying radiating arterioles and the draining collecting venules. As in the guinea pig and man the stria vascularis appears to be the cochlear vascular structure which is most difficult to inject regularly. This was most obvious in apical parts (Fig. 12).

Parallel and basal to the stria vascularis runs *the vessel of the spiral prominence* (Figs. 9 and 12). It runs more irregularly than in the guinea pig and is similar in appearance to the human cochlea. The radiating arterioles derive from the scala vestibuli and connect the vessel of the spiral prominence at rather short intervals. The radiating arterioles are of much larger calibre and occurrence than those supplying the stria vascularis (Figs. 9 and 12).

In the guinea pig and human cochlea most of the branches from the radiating arterioles do not supply the stria vascularis or the vessel of the spiral prominence but run externally to these vessels, forming direct *arterio-venous anastomoses*. The rabbit cochlea appears to be different in this respect since the arterio-venous anastomoses are more sparse (Fig. 12). This in turn seems to be due to the very rich supply to the vessel of the spiral prominence. Most of the branches of the radiating arterioles pass spirally for some distance in the region of the spiral prominence

before turning basally to the collecting venules of the scala tympani

Scala tympani

In the scala tympani there are two regularly occurring vascular structures.

Collecting venules
The venules at the basilar membrane

The collecting venules drain all the capillary areas of the external wall and are directly connected to the radiating arterioles by the above mentioned arterio-venous anastomoses (Figs. 9 and 12) Some large collecting venules drain the stria vascularis and others "protrude up to the scala vestibuli to drain the capillary net and the vessel at the vestibular membrane (Fig. 10) This was also observed by Nabeya (1923)

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In the *apical parts* of the cochlea the whole vascular pattern of the external wall is more open and sparse. Most of the regularly occurring vascular structures can also be demonstrated here (Fig 12). Stria vascularis is often difficult to inject completely The very pronounced supply to the vessel of the spiral prominence consequently appears still more prominently here The collecting venules of the scala tympani appear comparatively larger than more basally the largest drain the stria vascularis.

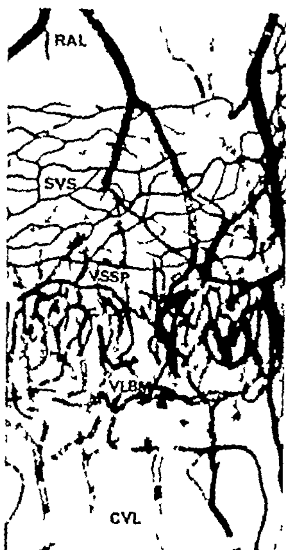


Fig 9 External wall basal turn Large radiating arterioles (*RAL*) supply all vessels and collecting venules (*CVL*) of the scala tympani drain all vessels. *SVS* – stria vascularis. *VSSP* – the vessel of the spiral prominence *VLBM* – the venules at the basilar membrane

Basal end

As in the guinea pig and human cochlea the basal end is very difficult to dissect so that the most basal portion the hook region can be photographically demonstrated. The appearance of this region between the windows is similar to that in guinea pig and man (Fig 13) The radiating arterioles and collecting venules take a more oblique course. The area of the round window (Fig 14) receives the

Fig. 10 External wall, basal turn. In the scala vestibuli wall there is a rich capillary net with a spiral basal vascular border the vessel at the vestibular membrane (*VSVM*). Both are drained by large collecting venules deriving from the scala tympani (*CVL*). *RAL* = radiating arterioles. *SVS* = stria vascularis.

short collecting venules in this region. From the vestibulum the radiating arterioles run in a retrograde apical direction to connect the radiating arterioles coming from the cochlear side just between the windows (Fig. 13) In this region the stria vascularis is particularly narrow and poorly developed. At the extreme basal end the stria vascularis again consists of more vascular loops (Fig. 13) Many of the branches of the radiating arterioles run to the vessel of the spiral prominence but comparatively many are also directed to the venules at the basilar membrane at the extreme basal end. At the extreme basal end there is a rich capillary net considerably more prominent than in the human and guinea pig cochlea.

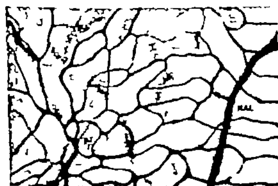


Fig 11 Stria vascularis, basal turn. The capillaries form open loops without any particular arrangement *RAL* = radiating arteriole.

DISCUSSION

For detailed information on the contrast injection and the vascular anatomy of the mammalian cochlea the reader is referred to the description of the guinea pig and human cochlea (Axelsson, 1968). The present description of the rabbit cochlea has instead been fo

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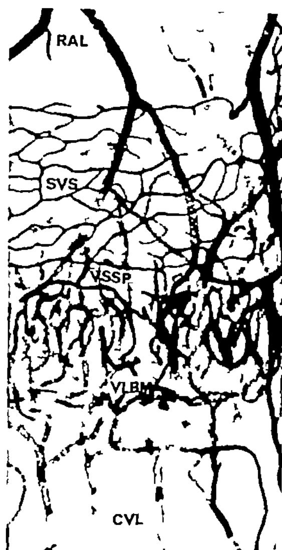


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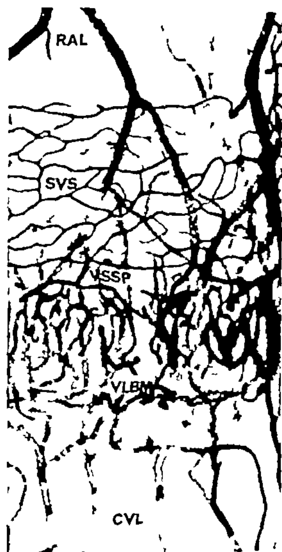


Fig. 9. External wall, basal turn. Large radiating arterioles (*RAL*) supply all vessels and collecting venules (*CVL*) of the scala tympani drain all vessels. *SVS* = stria vascularis. *VSSP* = the vessel of the spiral prominence. *VLBM* = the venules at the basilar membrane.

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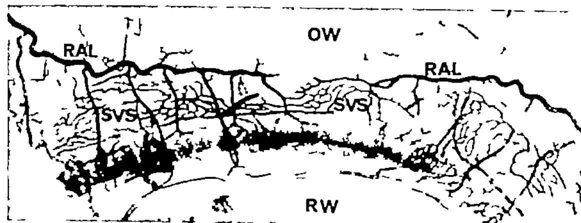


Fig. 13. Basal end. In the region between the oval window (*OW*) and the round window (*RW*) the radiating arterioles (*RAL*) coming from the cochlear side (*right*) merge with radiating arterioles coming from the vestibulum (*left*). Stria vascularis (*SVS*) appears narrow and poorly developed just between the two windows while both apically and basally to this area it contains many more loops, being particularly well developed at the extreme basal end (*left*).

Fig. 14 Basal end. The radiating arterioles (*RAL*) of the scala vestibuli and the collecting venules (*CVL*) emptying in the vein of the round window (*VRW*) take a more oblique course. Stria vascularis (*SVS*) is well developed. The vessel of the spiral prominence (VSSP) is not well injected in this preparation. *OW* = oval window *RW* = round window

The lack of apico-basally running collecting venules parallel to the radiating arterioles is well compensated for by the collecting venules of the scala tympani often "protruding" behind the stria vascularis to drain the capillary vessels of the scala vestibuli.

The vessel of the spiral prominence evidently must be particularly important for the rabbit cochlear function since it receives the majority of branches from the radiating arterioles.

Of the vascular structures in the spiral lamina the vessel of the basilar membrane has attracted most interest by previous investigators apparently due to its close proximity to the hair cells. It is well known that this vessel is much larger during embryonic life and invariably diminishes in size when the cochlea is fully developed (Hilding et al. 1967 Kikuchi & Hilding 1967). This indicates that the vessel of the basilar membrane may be more important for the development in the embryo than for adult function

The other capillary areas of the spiral lamina, i.e. the vessel of the tympanic lip and the limbus vessels, are very seldom described in comparison with the vessel of the basilar membrane. Lawrence (1971) omits both these vessels in a schematic drawing of the spiral lamina because "the vessels of the tympanic lip are sporadically appearing" This seems to be an underestimation. The vessel of the tympanic lip receives about 75% of all the supplying and draining vessels in the guinea pig and the vessel of the basilar membrane only 25% (Axelsson 1968) In animals where the vessel of the basilar membrane is missing there are always at least vascular arcades in the tympanic lip, in general forming a spiral vascular border This clearly demonstrates the importance of the vessel of the tympanic lip

The absence or degeneration of the vessel of the basilar membrane in animals with hereditary deafness referred to above has often been held responsible for the hearing defect. Strict precautions must be taken in drawing conclusions since this vessel may also be absent in normally hearing animals, e.g. cat, mink and rabbit. Since the vessel of the basilar membrane is missing in normal species the oxygen supply to the organ of Corti must obviously derive from other sources. The absence of this vessel makes the rabbit a particularly interesting animal for cochlear research. Possibilities are here available to compare the effect of induced changes in animals with and without a vessel under the organ of Corti.

Conclusions are often drawn from the appearance of the stria vascularis and the vessel of the basilar membrane concerning the complicated question of the oxygen supply to the cochlea. Apparently all regularly occurring vascular structures must be examined to allow any conclusions in this matter

ZUSAMMENFASSUNG

Das Gefässystem der Kaninchen-cochlea wurde mit Hilfe von Kontrastmittelinjektion dargestellt. Die Kapillarnetze sind denen der menschlichen und der Meerschweinchencochlea sehr ähnlich. Die Hauptunterschiede bestehen im Vorhandensein von spannenden Kapillaren in der Reissner'schen Membran und im Fehlen des Gefässe in der Basilarmembran (Vas spirale). Das Gefäss der Prominentia spiralis erscheint auf Grund seiner reichen Gefässversorgung als die wichtigste Kapillargefäss in der Aussenwand der Cochlea. Das Fehlen des Gefässes unter dem Cortischen Tunnelraum macht das Kaninchen besonders interessant für die Gehörforschung, insbesondere betreffs der komplizierten Frage der Sauerstoffversorgung des Cortischen Organs.

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Acta Otolaryng 76 268–272, 1973

SENSORI NEURAL HEARING LOSS IN JAMAICANS WITH SS DISEASE

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(Received January 16, 1973)

Abstract. The pure-tone hearing levels in 83 Jamaican patients aged from 10–39 with homozygous sickle cell disease were compared with appropriate controls. A sensori-neural hearing loss, defined as a deficit of at least 25 dB in one or more frequencies, was found in 18/83 (22%) patients and in 3/83 (4%) controls. Both sexes and ears were equally affected but the extremes of the tested range especially the high tones, were involved most frequently The hearing loss was of slow onset. The relative contributions of thrombosis, anaemia, atherosclerosis and Jamaican neuropathy are discussed. It is felt that the most likely aetiology is a thrombotic process.

Homozygous sickle cell disease (SS disease) is characterized by both a haemolytic anaemia and by episodes of vessel obstruction. The thrombotic process is considered to contribute to several clinical features of the dis- which include leg ulcers, priapism, avas- r necrosis of bone, pulmonary embolism, id apparently random infarctive lesions occurring anywhere in the body The organ of hearing is unlikely to be immune from this process and audiometry may act as a sensitive indicator of any sensori-neural (perceptive) hearing loss resulting from vascular damage.

Sensori-neural hearing loss was described in a 10 year old patient with "sickle cell anaemia (Morgenstein & Manace, 1969) in whom histological studies of the organ of Corti and the stria vascularis revealed changes consistent with ischaemia. In a review of sudden perceptive deafness, Morrison & Booth (1970) described one case with sickle cell-haemoglobin C disease and attributed this to a similar mechanism.

The incidence of sensori neural deafness in SS disease is unknown. Furthermore the relative contributions of anaemia and the thrombotic process to any hearing loss is also unknown. Evidence is accumulating that the irreversibly sickled cell (ISC) reflects the tendency to sickling in an individual with homozygous sickle cell disease, and so may be a useful parameter in studying this thrombotic tendency The present study was designed to investigate the incidence of sensori-neural deafness in a group of patients with SS disease and to assess the relevance of the anaemia (haemoglobin level), and of the thrombotic tendency (ISC) to these findings.

CLINICAL MATERIAL AND METHOD

The patients attended the adult sickle cell clinic at the University Hospital of the West Indies. *Four to eight patients were selected at random from each session of the weekly* clinic over a period from June to October 1971 Studies were performed on 83 cases with SS disease (40 male 43 female) with ages ranging from 12 to 39 years Patients above this age were excluded because of the pos-

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This work was supported by a grant from the Wellcome Trust London.

Table I. *Frequency distribution of hearing loss of 25 dB or more in left and right ears in SS and control groups*

Figures in parentheses refer to number of ears tested

	SS		Controls	
Frequency tested	Left ear (81)	Right ear (83)	Left ear (82)	Right ear (83)
500 Hz	5	2	0	0
1 kHz	0	0	0	0
2 kHz	0	3	0	0
4 kHz	4	4	0	0
8 kHz	8	7	1	3

sible effect of presbyacusis. All patients were in the steady state at the time of these studies.

The diagnosis of SS disease was based on the demonstration of only Hb S, F and A_2 on starch gel electrophoresis at pH 8.6 (Huehns & Shooter 1965). All cases had Hb A_2 levels below 3.6% as assessed by column chromatography (Huisman & Dozy 1965) in order to exclude cases of sickle β thalassaemia. Routine haematological methods were used as described elsewhere (Serjeant & Serjeant, 1972). Tests of treponemal serology using the Venereal Disease Research Laboratory Test, Reiter Protein Complement Fixation Test and the Fluorescent Treponemal Antibody Test were performed on 80 patients.

The control group contained 83 cases (40 male, 43 female) with ages ranging from 12 to 39 years, and matched for age and sex as closely as possible to the SS group. The controls were selected in an attempt to match the home environment. Thirty-two were neighbours, 18 were close relatives living in the same household, and the remaining 33 were friends accompanying patients attending the sickle cell or other clinics.

All patients were questioned regarding hearing loss, vertigo, and tinnitus with particular reference to the speed of onset, duration and course of these symptoms when present. Special attention was paid to the possibility of acoustic trauma as a result of explosions, gunfire, industrial noise and proximity to juke boxes and transistor radios (commonly applied directly to the ear in Jamaica).

A clinical otolaryngological examination including tests with tuning forks was performed. Hearing levels were assessed separately in each ear in a sound-proofed booth at the following frequencies; 500, 1 000, 2 000, 4 000, 8 000 Hz (Hertz—formerly given as cycles per sec). Measurements were made on a model A 17 Ambco audiometer calibrated to International Standards, and expressed in decibels (dB). Hearing levels below 500 Hz were not investigated because of a possible low-tone masking effect from an air conditioning unit in the room but outside the booth. However ambient noise level in the booth was below 30 dB as measured on the "A" scale on an Acos sound level meter.

A conductive hearing loss demonstrated by Rinne's Test and confirmed by audiometry occurred in the left ear of one control case (due to chronic suppurative otitis media) and in the left ear of two patients (one due to wax and the other to a traumatic deformity). Results of audiometry tests in these ears were excluded from the study.

RESULT

A history compatible with acoustic trauma was obtained in 8 patients. However the hearing characteristics of this group were similar to those with a negative history and in no case was the audiogram typical of acoustic trauma. It was therefore considered unlikely that acoustic trauma had contributed significantly to hearing loss and both groups were combined for subsequent analysis. The treponemal serology tests were positive in 15/80 (19%) patients. Preliminary analysis indicated that the hearing characteristics of patients with positive and negative tests were similar and so both groups were also combined for subsequent analysis.

Hearing loss, defined as a deficit of at least 25 dB in one frequency or more, was found

in 18/83 (22%) patients, and in 3/83 (4%) controls ($p = 0.001$). This difference remained highly significant when expressed as the number of ears or frequencies affected. Similar findings occurred in an analysis of hearing loss at the 10 dB or more level. Loss in the SS group occurred in both sexes and both ears equally. The extremes of the tested range were involved most frequently, with losses at 500 Hz in 7 ears, and at 8 kHz in 15 ears (Table I). Hearing loss in the control group was confined to 8 kHz (4 ears). In the SS group hearing loss was most common in the oldest group (30–39 years) and affected 27% ears (Table II).

There was a poor correlation between subjective deafness and objective assessments by audiometry. Of 18 patients with objective hearing loss, only 3 had noted impairment of hearing. On the other hand, 4 patients who considered themselves partly deaf had normal audiograms. In those who did notice a hearing loss, the onset was gradual, and unassociated with other aural symptoms such as vertigo or tinnitus.

No association could be demonstrated between hearing loss and the haemoglobin level or ISC count.

DISCUSSION

[Th]e significantly increased incidence of sensori-neural hearing loss in SS disease raises the problem of aetiology. Two pathological processes in SS disease which may be relevant are the anaemia and the role of thrombosis.

Anaemia has been proposed as an aetiological factor in sensorineural deafness for many years but there is little supporting evidence. Grant (1902) commented on anaemia as a cause of slow onset nerve deafness, and there have since been many references to chronic anaemic states in association with this type of hearing loss. However while certain haemorrhagic diatheses may be associated with a sensori-neural deafness of sudden onset, there is no data relating anaemia to a slow onset, slowly progressive hearing loss. Koide et al. (1961) reviewed studies of the effects of oxygen deprivation upon cochlear microphonics (a measurable electrical response of the cochlea to sound) and concluded that hypoxia led to widespread respiratory and circulatory problems before cochlear performance was affected.

Table II. *Age distribution of hearing loss in SS and control group*

	SS		Controls	
Age group (years)	No. of ears	Ears with hearing loss	No. of ears	Ears with hearing loss
10–19	81	12 (15%)	81	3 (4%)
20–29	57	4 (7%)	58	1 (2%)
30–39	26	7 (27%)	26	0 (0%)
Total	164	23 (14%)	165	4 ([illegible])

The haemoglobin level in SS disease is unlikely to reflect the oxygen carrying capacity since the decreased oxygen affinity of Hb S allows greater oxygen release per gram Hb than in Hb A. Furthermore the oxygen affinity is lower in cases with lower haemoglobin levels (Bromberg & Jensen 1967) thus making a simple relationship between haemoglobin level and oxygen carrying capacity unlikely. There was no association between hearing loss and the steady state haemoglobin level among SS patients in this study but failure to demonstrate a correlation does not exclude an effect related to hypoxia.

The cochlea receives its vascular supply through segmentally arranged arterioles with frequent anastomotic interconnections (Axelsson 1968). Vascular obstruction would therefore need to affect a larger vessel, such as the anterior inferior cerebellar artery to cause cochlear hearing loss. The resulting cochlear changes would be widespread, the hearing loss severe and the onset sudden (Perlman & Kimura 1957). This is not the pattern observed in SS disease. It is however possible that recurrent arteriolar obstructions impair the anastomotic communications and lead to small end artery lesions.

The principal venous drainage of the cochlea in both man and the guinea pig is the vena aqueductus cochleae. Occlusion of this vein in the guinea pig produces stagnation of cochlear blood flow, a decrease in endolymph oxygen tension, and a reduction in cochlear microphonics (Tsunoo & Perlman, 1964; Perlman, 1966). Occlusion for long periods causes permanent changes in both structure and function of the cochlea. The sites most commonly affected are the outer hair cells and the stria vascularis. The outer hair cells are considered to be the source of cochlear microphonics, the intensity of which diminish with degeneration of these structures (Davis et al. 1958). Kimura & Perlman (1956) noted that following experimental occlusion of the vena aqueductus cochleae, degeneration of the stria vascularis and outer hair cells occurred in a regular sequence. The basal turn was initially affected (6 days), followed by the apical turn (2 months) and finally the whole of the cochlear duct (5 months). The sensitivity of the basal turn of the cochlea to anoxia is considered due to the high oxygen consumption rate of the stria vascularis in this area and to the poor capacity for anaerobic metabolism (Koide et al. 1964; Conti & Borgo, 1964). The basal turn of the cochlea records high frequencies, and the apical turn low frequencies, so that in experimental venous obstruction, it might be expected that high frequencies would be first affected, followed by low frequencies, and ultimately deterioration at all frequencies. This is similar to the pattern of hearing loss in SS disease and it is possible that a low grade continuous venous thrombotic process, without clinically recognised episodes, affects the cochlea in a similar manner.

Such a mechanism probably causes the progressive splenic fibrosis characteristic of sickle-cell anaemia. The lack of a correlation between irreversibly sickled cells (ISC) and sensori-neural deafness in the present group does not give support to this aetiology but only small numbers were available for analysis. ISC counts were not available in 3 of the 18 cases with hearing loss. It is likely that factors such as age also contribute to the hearing loss noted in this disease, and a further statistical analysis on much larger numbers would be required before an effect of ISC can be excluded.

Atherosclerosis has also been suggested as a cause for hearing loss but the evidence is conflicting. Weston (1964) stated that atherosclerosis was a factor determining the hearing level of elderly subjects, but Bunch (1931) found no difference in the hearing levels between atherosclerotic and non-atherosclerotic groups of patients. Studies in diabetic populations have also revealed conflicting results. Hearing impairment has been noted in several surveys, but a recent investigation of diabetics and controls (aged 16–50 years) with pure-tone audiometry has indicated good preservation of function in the diabetic group (Axelsson & Fagerberg 1968). The low incidence of atherosclerosis in Jamaicans (Gore et al., 1960) and at this age group makes it unlikely that this mechanism has contributed significantly to hearing loss in the present series.

Other possible aetiologies of hearing loss are speculative. Compensatory bone marrow hypertrophy in SS disease causes expansion and deformity of some marrow containing bones including the temporal bone (Morgenstein & Manace 1969; Grinnan 1935). It is conceivable that bone expansion narrows the internal auditory canal leading to pressure on the eighth cranial nerve. With slow neural compression, hearing loss may occur without obvious disturbance of vestibular function, and progressive hearing loss by this mechanism is well recognised in Paget's disease. Tomography of the internal auditory canal in patients with SS disease and hearing loss might reveal evidence of bony overgrowth compatible with neural compression.

A sensori-neural hearing deficit of unknown aetiology has been described in association with peripheral neuropathy (of spastic

and ataxic types) and retrobulbar atrophy in Jamaica (Montgomery et al. 1964). However there were no neurological signs associated with hearing loss in the present study and a "forme fruste" of this neurological syndrome producing only hearing loss might be expected to affect controls as well as patients.

The interest of hearing loss in SS disease lies not in the symptomatology of the disease since subjective hearing loss is rare, but in the mechanism of aetiology. Future studies with more detailed audiometric tests (e.g. Békésy, Loudness recruitment), tomography of the internal auditory canal, and pathological examination of the cochlea and temporal bone may allow more precise localization of the lesion.

ACKNOWLEDGEMENT

We would like to thank the Medical Research Council Epidemiology Unit, University of the West Indies (A. Davis, M.D., F.R.C.P.E., D.T.M. & H., Mrs P Desai, B.A.), and Dr R. Hinchcliffe, Ph.D., B.Sc., M.R.C.P., M.R.C.S., D.L.O., of the Institute of Laryngology and Otology for their help and advice.

ZUSAMMENFASSUNG

e reine Tonhöhe on 83 jamaikanischen Patienim Alter von 10–39 Jahren, mit homozygotischer ...enanämie wurden mit entsprechenden Kontllen verglichen. Ein Nervenhörverlust, ausgedrückt als ein Verlust on wenigstens 25 dB in einer oder mehreren Frequenzen, wurde in 18 Fällen bei den 83 Patienten (..2) und in 3 bei den 83 Kontrollen (4) gefunden. Beide Geschlechter und beide Ohren waren gleichmässig beteiligt. Am meisten waren die oberen und unteren Grenzwerte besonders die der hohen Töne betroffen. Der Hörverlust setzte langsam ein. Der jeweilige Einfluss on Thrombose, Blutarmut, Arteriesklerose ist besprochen worden. Es wird angenommen, dass die Ursache sehr wahrscheinlich ein thrombotischer Prozess ist.

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Acta Otolaryng 76 273–276 1973

EINE TESTANORDNUNG FÜR DAS RICHTUNGSHÖREN IN DER VERTIKALEBENE

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(Eingegangen am 1 Februar 1973)

Abstract. Der Fähigkeit der Schallokalisation in der Vertikalebene liegt ein anderer physiologischer Mechanismus zugrunde als der Untersuchung in der Horizontalebene. Die Versuchsanordnung besteht aus 9 Lautsprechern, die in einem Abstand von 2,5 m vor dem Patienten angeordnet sind. Durch Verdecken eines Ohres ist eine monaurale Untersuchung gegeben. Die Fehlerzahlen des rechten und des linken Ohres zeigen beim normalen Probanden keinen signifikanten Unterschied. Bei dieser Untersuchung ist einerseits die Ohrmuschel von großer Bedeutung, da sie die Klangfarbe mit dem Einfallswinkel des Schalles ändert. Andererseits liegt vermutlich eine Gedächtnisleistung vor — das Erinnerungsvermögen für die Klangfarben. Es scheint daher von Interesse zu sein, Patienten mit cerebralen Schädigungen zu untersuchen.

Die akustische Orientierung in der Vertikalebene, der Horizontalebene und der Tiefe des Raumes müssen getrennt betrachtet werden. Die subjektiven Räume des Menschen sind wahrscheinlich keine Euklidischen Räume, für die die drei Ebenen gleichwertig anzusehen sind. Spezielle Untersuchungen für den visuellen Raum (Kienle, 1968) haben ergeben, daß hier die Gesetze der hyperbolischen Geometrie (Bolyai & Lobatschefsky 1947) anzuwenden sind. Eine wesentliche Informationsquelle fur den subjektiven akustischen Raum ist die Fähigkeit der Schallokalisation. Dieser Raum ist schon deshalb nicht als euklidisch anzusehen, da diese Lokalisation für Horizontal- und Vertikalebene verschiedene physiologische Mechanismen benützt. Für das Richtungshören in der Horizontalebene gelten vor allem die Faktorengruppe Laufzeitdifferenz, Phasen- und Intensitätsunterschied (Jongkees & Veer 1958 Nordlund, 1964) während für die Vertikalebene vor allem die von der Ohrmuschel bewirkte Klangfarbenänderung zusammen mit einem Gedächtnisfaktor maßgebend ist (Bolle et al., 1948 *a* *b* König & Sussman, 1955). Die der Lokalisationsfähigkeit in *der Tiefe des Raumes* zugrundeliegenden Mechanismen sind noch zu wenig untersucht, beruhen aber abgesehen von der Lautstärke möglicherweise auch auf einer Änderung der Klangfarbe durch die Ausbreitung der Schallwelle im Raum hinzu kommt unter normalen Bedingungen die Reflexion (Tabelle I.) Ein Grenzproblem des Richtungshörens stellt die Unterscheidung von vorne und hinten am Schnittpunkt der sagittalen Vertikalebene mit der Horizontalebene dar (Kietz, 1952).

METHODIK

Sicher sind die Probleme der räumlichen Orientierung in der Vertikalebene schwer erfaßbar als dies bei den Versuchsanordnungen für die Horizontalebene der Fall ist. So ist es kaum möglich einen Grenzwinkel zu bestimmen. die Angaben der Versuchspersonen widersprechen einander und das Ergebnis ist daher unsicher. Zeitweise zeigt der Proband eine gute Orientierung, dann wieder werden wesentlich größere Abweichungen von der Mitte nicht

Tabelle I. *Faktoren, die das Richtungshören beeinflussen und ihre Wertigkeit für die drei Ebenen des Raumes*

Von einigen Autoren wurden weiters die Knochenleitung sowie das vestibuläre System angeführt (hier weggelassen)

	Vert.	Horiz.	Tiefe
Laufzeit	0	+ +	?
Phasendifferenz	0	+ +	?
Intensitätsunterschied	0	+ +	+
Kopfbewegungen	+	+	?
Zentraler Richtungseffekt (Erfahrung)		+	+
Veränderung der Klangfarbe			
Ohrmuschel	+	+ ?	
Reflexion	?	?	?
Lauf in der Luft	?		+ ?
Tonhöhe	?	?	+ ?
Tondauer (bei kurzen Stimuli)	?	?	?

erkannt. Wir haben es vorgezogen, auf die Bestimmung des Grenzwinkels zu verzichten und ermitteln statt dessen ein Ergebnis, das dadurch gewonnen wird, daß verschiedene Lokalisationen in statistischer Reihenfolge angeboten werden. Es werden also Positionen, die nur relativ wenig von der Mittellinie abweichen, abwechselnd mit den weiter ent[illegible]n Lautsprechern angeboten und da[illegible]chen ertönt immer wieder der mittlere [illegible]echer (s.u.), der Proband hat also durch den unterschiedlichen Abstand vom Mittellautsprecher neben schwierigen Positionen immer wieder die Möglichkeit einer annähernd eindeutigen Orientierung. Der Abstand der Lautsprecher von der Mittellinie ist so gewählt, daß kein Proband alle angebotenen Positionen richtig bezeichnen konnte, andererseits werden bei schlechter Orientierung die extrem weit oben bzw. unten angebrachten Lautsprecher meist erkannt. Ob noch eine Orientierung vorhanden ist, erkennt man durch den Vergleich mit einer statistisch errechneten Fehlerzahl (134), die einer völligen Orientierungslosigkeit entspricht. Die Anordnung (Abb. 1) besteht demnach aus 9 Lautsprecher, die übereinander in einem Kreisbogen angeordnet sind, der Abstand vom Pat. beträgt 2,5 m, der mittlere Lautsprecher ist in Kopfhöhe des Patienten, der Winkel zwischen den einzelnen Lautsprechern 4,5°. Bei den Lautsprechern konnte kein Unterschied in der Klangfarbe festgestellt werden. Die Anlage ist zur Vermeidung von Reflexionen in einer Camera silens untergebracht. Als Geräuschquelle dient Schmalbandrauschen eines Viennatone-Audiometers (M 132), da sich Rauschen mit seinen Unregelmäßigkeiten viel besser als Sinustöne für die Verformung durch die Ohrmuschel eignet (Roffler & Butler 1968). Das akustische Signal wird aus Gründen einer besseren räumlichen Orientierung immer zuerst für die Dauer von 1 sec. über den mittleren Lautsprecher und nach einer Pause von 1 sec. wieder für 1 sec. über einen der anderen Lautsprecher angeboten, wobei diese 8 Lautsprecher in statistischer Folge abgewechselt werden. Die Signale und Pausen werden automatisch mittels elektronischer Relais geschaltet. Die Versuchsperson muß angeben, welcher der 8 Lautsprecher als zweiter ertönt. Um den Probanden an die Versuchsbedingungen zu gewöhnen, wird zunächst binaural stimuliert, danach wird jeweils ein Ohr durch WN über einen Kopfhörer verdeckt. Zunächst werden 24 Positionen dem linken Ohr angeboten, dann 24 Positionen dem rechten Ohr. Nach einer Ruhepause wird die Prüfung in umgekehrter Reihenfolge wiederholt. Der Abstand zwischen dem tatsächlich angebotenen und dem vermuteten Lautsprecher wird als Fehler bezeichnet, wobei jeder Winkel von 4,5° (Abstand zweier Lautsprecher) als Fehlerpunkt gilt. Die Addition ergibt die Gesamtfehlerzahl, getrennt für beide Ohren (Fritze & Gloning, 1971).

ERGEBNISSE

Schon die binaurale Prüfung zeigt, daß eine relativ große interindividuelle Streuung besteht, wobei wir keine Abhängigkeit vom Intelligenzquotienten finden konnten. Weiters nehmen die Fehlerzahlen mit zunehmendem Alter stark zu. Es zeigte sich auch, daß jede

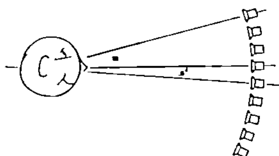


Abb. 1 Neun Lautsprecher sind in der sagittalen Vertikalebene 2,5 m vor dem Patienten aufgestellt.

Veränderung der Ohrmuschel das Ergebnis verschlechtert, was auch von Kietz (1952) beschrieben wurde. Ferner fanden wir daß die Lautstärke keinen wesentlichen Einfluß auf das Testergebnis hat, wenn man den nur knapp überschwelligen Bereich unberücksichtigt läßt. Die subjektive Hörschwelle ist nicht von so großer Bedeutung wie bei Versuchen in der Horizontalebene. Schalleitungsstörungen haben einen relativ geringen Einfluß Schallempfindungsstörungen verschlechtern das Ergebnis wesentlich.

Bei allen normalen Versuchspersonen wich die Fehlerzahl des rechten Ohres von der des linken Ohres nicht signifikant ab (χ^2-Probe, Hofstätter & Wendt, 1966.) Die erzielten Fehlerzahlen schwanken also interindividuell u. innerhalb der Altersgruppe, sind jedoch bei getrenntohriger Untersuchung innerhalb der statistischen Streugrenze. Daher ist diese Untersuchungsmethode vor allem für den Seitenvergleich geeignet.

Die Ergebnisse von 52 normalen Versuchspersonen, getrennt nach Händigkeit, sind in Tab II dargestellt. Die mittels Varianzanalyse untersuchten Fehlerzahlen der einzelnen Gruppen zeigten keinerlei signifikanten Unterschied, es besteht nicht einmal ein Trend. (Gloning et al. 1954). Die Gruppe von blinden Versuchspersonen wurde deshalb in die Untersuchung einbezogen, da bei fehlender visueller Orientierungsmöglichkeit Komponenten der akustischen Orientierung besser ausgebildet sein könnten. Es zeigte sich jedoch — wie es von Pruszewicz & Gerwel (1964) für die Horizontalebene beschrieben wurde — daß blinde Versuchspersonen keine bessere Orientierungsfähigkeit in der Vertikalebene besitzen.

Tabelle II

a. *Die Fehlerzahlen der Gruppen Rechtshänder Linkshänder und Blinde*

Es zeigt sich kein signifikanter Unterschied, (Varianzanalyse). Auch Blinde haben keine niedrigeren Fehlerzahlen

	Rechtes Ohr		Linkes Ohr	
	$\bar{X}$	s	$\bar{X}$	s
Rechtshänder ($N=27$)	57 48	19,93	57,63	20,08
Linkshänder ($N=25$)	57,52	23,76	56,76	25,41
Blinde ($N=10$)	57,56	4,08	57,56	-1,53

b *Das durchschnittliche Alter der Probanden*

	$\bar{X}$	s
Rechtshänder	26,44[b]	11 42
Linkshänder	30,00	11,55
Blinde	20,00	18,07

$\bar{X}$ Mittelwert. s Streuung.

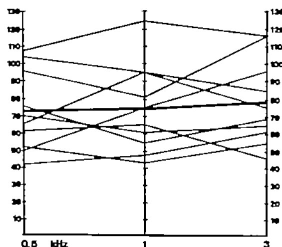


Abb 2 Die Unterschiede zwischen drei Frequenzen bei der Testung des rechten Ohres von 10 Versuchspersonen. Die dicke Linie gibt den arithmetischen Mittelwert an, das Verhältnis der Fehlerzahlen trägt 73,0 75,3 79,0 für 0,5 1 3 kHz.

Bei der monoauralen Untersuchung kann man einzelne Frequenzen miteinander vergleichen, was bei Verwendung von Schmalbandrauschen möglich ist. Es zeigte sich jedoch nur ein sehr geringfügiges Ansteigen der Fehlerzahl zu den hohen Frequenzen (Abb 2).

DISKUSSION

Laufzeit Phasen- und Intensitätsunterschiede werden in verschiedenen Stationen der Hörbahn verglichen. Störungen dieser akustisch-räumlichen Koordination (Horizontalebene) durch verschiedene cerebrale Prozesse wurden unter anderem von Matzker (1959) Nordlund (1964) sowie von Szikazay & Gloning (1969) beschrieben.

Es bleibt nun zu untersuchen inwieweit Störungen des Richtungshörens in der Vertikalen — im Sinne der oben beschriebenen Versuchsanordnung — mit cerebralen Läsionen zusammenhängen denn es ist zu vermuten daß bei bestimmten Großhirnschädigungen, vor allem der Hörrinde und ihrer Umgebung, der Gedächtnisfaktor dieser Leistung — das Erinnerungsvermögen für Klangfarben — einer Störung unterliegt.

* G Divis sind wir für die wertvolle Mithilfe bei der Durchführung der Untersuchungen zu Dank verpflichtet.

SUMMARY

The faculty of sound-localization in the vertical scale is based on other physiological mechanisms than the distinction in the horizontal scale. The test-situation consists of 9 loudspeakers, arranged one above the other at a distance of 2.5 meters in front of the patient. Narrow-band noise is emitted in turn from the middle and from one of the other loudspeakers. By covering one ear a monaural test is provided. The test results obtained from the right and left ear of a normal person show no significant difference. At this test the ear mould is of great influence, since it changes the timbre of the sound according to the angle of incidence. An ability to remember is also presumed—the memory for the timbre. For that reason it would seem to be of interest to investigate patients with cerebral lesions.

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Acta Otolaryng 76 277–282, 1973

VASODILATATION IN THE NASAL MUCOSA OF THE CAT AND THE EFFECTS OF PARASYMPATHOLYTIC AND β ADRENERGIC BLOCKING AGENTS

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(Received January 31 1973)

Abstract Stimulation of the vidian nerve in cats within a certain range of intensities evokes an increased venous flow from the nose and a decreased nasal patency These effects are assumed to be due to vasodilatation, which is highly resistant to the parasympatholytic agent, piperidino-ethyl-diphenyl-acetamide (Hoechst 9980). The vasodilatation was not significantly changed at stimulation after the combination of Hoechst 9980 and a β-adrenergic blocking agent, propranolol. Possible vasodilator mechanisms are discussed.

In the cat, the vidian nerve consists of post ganglionic sympathetic and preganglionic parasympathetic nerve fibres. The latter fibres synapse in the pterygopalatine ganglion and the two sets of postganglionic nerve fibres innervate the vessels and supposedly the glands in the nasal mucosa (Malcomson, 1959) Many investigators have reported that the effects of sympathetic stimulation of the nasal mucosa in dogs and cats are blocked by α-adrenergic blocking agents (Malcomson, 1959· Hall & Jackson, 1968 e g.). The effects of stimulation of the preganglionic parasympathetic fibres are known to be prevented by ganglionic blocking agents (Tschalussow 1913), but no reports are available about parasympathetic stimulation after parasympatholytic agents. A method has been described (Malm 1973), where venous flow from one nose cavity in the cat is recorded simultaneously with nasal patency Stimulation of the vidian nerve within certain intensities and frequencies evokes an increased venous flow together with a decreased nasal patency and this is taken as evidence of vasodilatation. In this investigation the vasodilatation was studied by electrical stimulation of the vidian nerve in the cat before and after parasympatholytic and β-adrenergic blocking agents.

METHOD

The experiments were made on 9 cats under chloralose anaesthesia using the methods described in a previous paper (Malm, 1973). Venous flow from one nose cavity was obtained from a vein emerging from the pterygopalatine foramen and was recorded as drops of blood by an ordinate writer on a smoked drum. Simultaneously the pressure changes in a water-filled balloon in the same cavity were recorded on a polygraph. The resting pressure was 6 cm of water above the nose cavity When the vessels in the pterygomaxillary fossa were dissected under microscope, care was taken to tie the small vein from the zygomatic gland because this gland may be innervated by the vidian nerve. The peripheral end of the cut vidian nerve was stimulated with a bipolar electrode at a duration

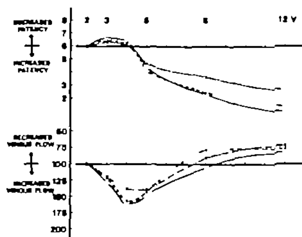


Fig. 1. Mean responses from 8 cats to stimulation of the vidian nerve at a frequency of 10/sec and at various intensities before Hoechst 9980 (o—o) after Hoechst 9980 (Δ- Δ) and after Hoechst 9980 and propranolol ( ) intravenously. *Ordinates from above*: pressure in the balloons in cm of water above the nose, changes in the venous flow in percentage of the resting flow. *Abscissa*, stimulation intensity (volts).

of 3 msec, at a frequency of 10 per sec and at various intensities.

Acetyl-β methylcholine chloride (methacholine) atropine sulphate piperidino-ethyl diphenyl-acetamide (Hoechst 9980) isopropyl-noradrenaline sulphate (isoprenaline) and propranolol hydrochloride were given intravenously

RESULT

Previous experiments have shown (Malm 1973) that when the venous flow from the nose and the nasal patency are investigated under the experimental conditions described, the threshold intensity of the preganglionic parasympathetic nerve fibres is about 3 volts and of the postganglionic sympathetic about 4 V. Even if both types of nerves are activated from 4 V and upwards the parasympathetic effects on the venous flow are dominant up to about 8 V. A decreased nasal patency appears, however only at 3 and 4 V. These observations, which were discussed in a previous paper (see Malm, 1973) are seen in Fig. 1 where the mean values of 8 cats are given as solid lines. The results from one of the cats are also demonstrated in Fig. 2, where it can be noted that when stimulation ceased the venous flow and the nasal patency generally returned to resting values in less than half a minute.

Stimulation of vidian nerve after parasympatholytic agents

After stimulation of the vidian nerve as described above all the cats were given a parasympatholytic agent. One cat received atropine and 8 cats Hoechst 9980, which is highly specific as an antagonist to the muscarinic effects of acetylcholine (Schaumann & Lindner 1951).

Some of the cats were first given 0.1 mg/kg i.v. of the parasympatholytic agents because this dose blocks secretory responses evoked by parasympathetic nerves to salivary glands (Emmelin & Henriksson, 1953). Stimulation changed the venous flow and the nasal patency in the same direction and to the same degree as before the parasympatholytic agents were given. Then all the cats were given 1 mg/kg i.v. *This dose eliminated* the effects both on the nasal mucosa and on the blood pressure of the intravenously given parasympathomimetic drug methacholine. When the vidian nerve then was stimulated using various intensities the effects on the venous flow and the nasal patency in all cats were nearly the same as before the parasympatholytic agents. The mean differences of paired values at stimulation before and after the 8 cats were given Hoechst 9980 were small and for the venous flow at 3 V 6±5% and at 4 V 4±5% and for the nasal patency 0.2±0.1 and 0.1±0.1 cm of water respectively. The mean values are given in Fig. 1. Fig. 3 represents the continuation of the records from the same cat as in Fig. 2.

The immediate effect of Hoechst 9980 and atropine was a blood pressure rise, a decrease of the venous flow and an increase of the nasal patency in 8 of the 9 cats. In the remaining cat which was one of the cats which

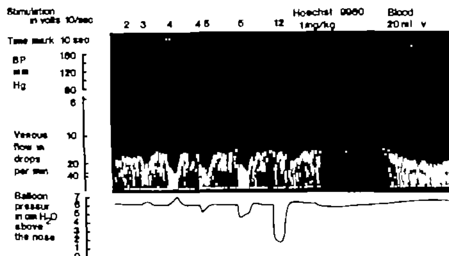


Fig. 2. Cat, 3.1 kg. Responses to stimulation at various intensities and a frequency of 10/sec of the peripheral end of the cut vidian nerve before Hoechst 9980 was given. The effects of Hoechst 9980 and blood intravenously are also seen. Stimulation periods and injections (signals upwards) are marked underneath time record. Lowest record represents balloon pressure transposed from the polygraph record. A decreased balloon pressure marks an increased nasal patency

had received Hoechst 9980 the venous flow and the nasal patency did not change but the blood pressure rose. As a blood pressure rise of about the same magnitude evoked by blood or saline intravenously always increases the venous flow and decreases the nasal patency (see Fig. 2) the effects of the parasympatholytic agents *per se* seem to be decreased venous flow and increased nasal patency

Stimulation of vidian nerve after a β-adrenergic blocking agent

Gautvik (1970) has demonstrated that propranolol is able to reduce the vasodilatation in salivary glands after stimulation of the

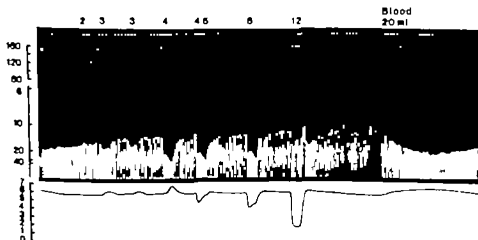


Fig. 3. Continuation of Fig. 2. Responses to stimulation after Hoechst 9980 has been given. Stimulation frequency was as in Fig. 2, 10/sec and intensities as indicated.

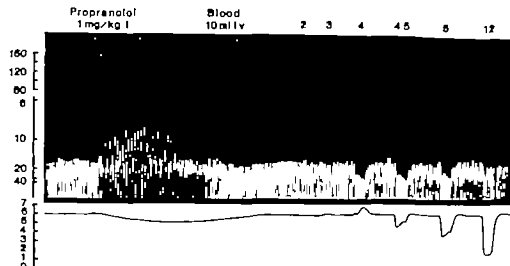


Fig. 4. Continuation of Fig. 3. The effects of propranolol are first seen. The responses to stimulation after Hoechst 9980 and propranolol are then given. The frequency and the intensities were as in Fig. 2.

parasympathetic nerves and has shown that it decreases the formation of bradykinin. In order to investigate if such a blocking agent has an effect in the nasal mucosa, the cats, which had received Hoechst 9980, were given 1 mg/kg propranolol intravenously. The immediate effect was a decrease of the blood pressure, a decreased venous flow from the nose and an increased nasal patency. About an hour later when the blood pressure had returned to normal or nearly normal values, the vidian nerve was stimulated as before at various intensities. The mean differences of paired values at stimulation before and after the 8 cats were given Hoechst 9980 and propranolol were for the venous flow at 3 V 10±9% and at 4 V 22±8% and for the nasal patency 0.3±0.1 and 0.3±0.2 cm of water respectively.

The mean differences were greater than after Hoechst 9980 alone but they were still not significant. The mean values are given in Fig. 1. Fig. 4 represents the continuation of the records from the same cat as in Figs. 2 and 3 and it can be seen that after Hoechst 9980 and propranolol the venous flow and the nasal patency at stimulation return to resting values in about the same time as before.

The effects of intravenously given methacholine both on the nasal mucosa and the blood pressure could be eliminated by Hoechst 9980. The pressure fall, caused by isoprenaline, was found to be abolished by propranolol in the time period during which the stimulation experiments on the vidian nerve were done. The effects of isoprenaline on the nasal venous blow and patency could, however not be eliminated, only reduced by propranolol. This relative resistance to propranolol, which has been demonstrated in nasal mucosa of dogs by Hall & Jackson (1968), will be subjected to a later investigation in cats.

DISCUSSION

Vasodilatation when stimulating parasympathetic nerves can, for instance, be seen in salivary glands. It was a matter of discussion for many years if the increase of blood flow is due to activation of vasodilator nerve fibres or to formation of a vasodilator agent (Hilton, 1960; Schachter, 1969). Gautvik (1970) has, however demonstrated two phases of vasodilatation on the submaxillary gland of the cat. One of the phases is brief and is assumed to be due to activation of vasodilator nerve fibres, the other is longlasting and at

tributed to release from glandular cells of a "bradykinin-releasing enzyme" called kallikrein and formation of a vasodilator polypeptide, bradykinin or kallidin. The nasal mucosa is richly supplied with glandular cells and it seems possible that vasodilatation there too can be evoked by the two mechanisms.

Heidenhain (1872) and many investigators after him found in experiments on salivary glands that the vasodilatation caused by stimulation of parasympathetic nerves is highly resistant to atropine. A similar resistance of the vasodilatation in the nasal mucosa towards parasympatholytic agents can be seen in the present experiments. When the vidian nerve was stimulated within a certain range of intensities atropine and Hoechst 9980 seemed to have no or very little effect on the vasodilatation.

The symptoms of vasomotor rhinitis in man are sneezing, watery secretion and nasal congestion. During attacks an overactivity in the parasympathetic nerves is supposed to occur (Golding Wood, 1961). If it is so and if parasympatholytic agents have as little ability to prevent vasodilatation in man as in the cat at an increased activity in parasympathetic nerves, these agents ought to be of no use against the nasal congestion in vasomotor rhinitis. It may be worth while, however to investigate if the small effects of decreasing venous flow and increasing nasal patency which the parasympatholytic agents were found to exert and which were not further analysed, occur in humans also.

Davey et al. (1965) suggested that adrenergic β-receptors play a role in the chorda mediated vasodilatation in the submaxillary gland of the cat. Skinner & Webster (1968) reported that the combination of propranolol and atropine could reduce the chorda-evoked vasodilatation more than each drug alone. Gautvik's (1970) experiments on the same gland did not support the view that β receptors are important for the vasodilatation caused by vasodilator nerves, but he found that the combined action of propranolol and atropine affected the kinin-dependent vasodilator mechanism. According to the present experiments propranolol given after Hoechst 9980 did not change the magnitude of the vasodilatation significantly but there was a tendency to reduction. To take this as evidence of the existence of a kinin-dependent vasodilatation in the nasal mucosa may however be premature

ZUSAMMENFASSUNG

Bei Reizung mit bestimmten Intensitäten des Nervus Vidianus von Katzen erhält man eine erhöhte venöse Strömung von der Nase aus und eine Nasenschleimhautanschwellung. Es wird angenommen, dass diese Effekte eine Vasodilatation ist, die ausgesprochen resistent gegenüber der parasympatolytischen Substanz piperidino-ethyl-diphenyl-acetamide (Hoechst 9980) ist. Die Vasodilatation war bei Reizung nach einer Kombination von Hoechst 9980 und einem β-Blocker Propranolol, unbedeutend verändert. Eventuelle vasodilatatorische Mechanismen werden diskutiert.

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Acta Otolaryng 76 283–294 1973

SCANNING ELECTRON MICROSCOPY OF THE NASAL MUCOUS MEMBRANE

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(Received January 13 1973)

Abstract. The structures of components of the nasal mucous membrane have been studied by means of the scanning electron microscope. The mucous covering the lining epithelial cell as well as intercellular ground substance in the subepithelial layer has been clearly visualized. The following patterns of the structures have been characterized in this study Noodle-like shape of cilia of the ciliated cell; club-like process of microvilli of the goblet cells; polygonal shape with numerous microvilli of the basal cells; bundles and meshworks of the connective tissue fibers; numerous pores on the inner wall of the venules; microvilli and secretory granules of the inner surface of the terminal portion of the secretory glands, and spiral pattern of the inner wall of the secretory ducts.

A number of studies of the structures of the nasal mucous membrane have been made by means of the light microscope and the transmission electron microscope. Even though the structures, including the normal and involved condition, are well known, many problems concerning their detail remain to be studied. The purpose of the present paper is to demonstrate the histologic pictures of the components of the nasal mucous membrane such as the cilia, microvilli, epithelial lining cells, the vessels, connective tissue fibres, ground substance, the secretory gland, and duct.

Both rabbit and human specimens were examined in three-dimensional visualization by means of the scanning electron microscope as described by the present authors in a previous paper To date, studies using animals have mostly examined the cilia of the nasal mucous membrane (Barber & Boyde 1968 Graziadei, 1970) The present study also shows that the introduction of the scanning electron microscope into biological research has revolutionized the study of surface structures of biological specimens, as described by Barber

MATERIAL AND METHOD

Specimens examined in the experiment were as follows:

Sample 1 A small piece of human nasal polyp as well as the mucous membrane of the lower border of normal or affected inferior turbinate, were obtained by punch forceps for biopsy without any local anesthesia, in order to avoid injury to the tissue.

Sample 2 The ciliated cells were isolated by scratching the surface of the normal inferior turbinate in the human. In this way the curette for the nasal operation made ran superficially and gently along the surface so as not to be associated with pain and bleeding. The sample obtained, consisting of the mucous, lining epithelial cells, leucocytes, and so on, was then rinsed in saline and placed on the surface of a coverglass, being subsequently disintegrated with the back of the curette. Thereafter they were allowed to air dry

Sample 3 White rabbits, weighing approximately 2 kg were killed by air injection and

the anterior portion of nasal septum including the cartilage was removed.

Sample 4 A small piece of rabbit nasal septum obtained by the same method as in sample 3 was scratched on the surface on one side, and the overlying mucous layer and lining epithelia were removed by the same method as in sample 2.

Both specimens thus obtained were rinsed and washed clear of blood after which the mucous covering the specimen surface was shaken gently and repeatedly in saline until the saline remained clear Samples 1 3 and 4 were then cut vertically down to the epithelial surface with a razor blade, forming 5 mm squares (approx.) All samples, including no. 2, were fixed in cold 4% cacodylate buffered glutaraldehyde for 4 hours in the refrigerator The fixed specimens were kept in cold 0.05 M cacodylate buffer solution until required. They were then dehydrated in graded acetone solutions of 70% 80% and 99% followed by two changes of absolute acetone, and allowed to air dry

Some specimens of sample 1 were transferred to amylacetate after dehydration in the acetone series and dried by the critical point method according to Boyde. The cilia these specimens were examined. The dried cimens were mounted with adhesive silver aste on specimen carriers in such a way that one had the free epithelial surface of the mucous membrane up and another had one side of the sectioned surface vertical to the epithelial surface up, in each of samples 1 3 and 4 The surfaces of the mounted specimens were then thinly vacuum-coated with as little as approx 200 Å of carbon-gold in order to render them electrically conductive The specimens made were examined on the specimen stage at an angle of 45 at between 5 and 20 kV at accelerating voltages by JSM U3 scanning electron microscope (Japan Electron Optics Lab. Co) A part of each sample obtained was also prepared for histopathological study by light microscope Paraffin embedding and subsequent routine preparation of the tissue by usual histologic technique was performed. Another part of the same specimen was examined by transmission electron microscope after fixation in 2% OsO in cacodylate buffer embedding in Epon 812 sectioning and staining in uranyl acetate and lead citrate

RESULT

1 There seem to be two kinds of mucous covering the surface of the mucous membrane, differing in the ease with which they can be washed out by saline rinsing. One type which adheres to the tips of groups of cilia, is very difficult to remove completely the other which fills the spaces among the cilia, is easier to wash out (Fig. 1).

2. The individual cilia seen are approximately 5 μm in length and 0.2 μm in width, and appear dull at the tip uniform in size at the shaft and have numerous fine stripes on the free surface. Numerous cilia from one cell are grouped and wave in a certain direction together with the adjacent groups of cilia although each cilium of one cell showed metachronal motion (Figs. 2, 3 4)

The isolated ciliated cell of sample 2 tapers off at its proximal portion and is seen to be rougher on the outer surface than when seen by transmission electronmicroscope (Fig. 5).

3 A number of groups of microvilli were seen among the groups of cilia. On the free surface of the specimen of the nasal polyp, cell boundaries of groups of microvilli were discriminated. All microvilli, crowded and regularly arranged on the cell surface were of short club-like appearance and approximately 500 in number per cell. The cell with microvilli was presumed to be a goblet cell (Fig 6).

4 Another type of lining epithelial cell was seen on the free surface of affected mucous membrane instead of the ciliated epithelium. This was elongated or rounded in shape, rough on the surface and loose in connection to the adjacent cells. These cells were



Fig. 1 Normal human inferior turbinate. 10 000. Free surface of the mucous membrane. By rinsing in saline the mucous covering the surface of the mucous membrane is partly lost, and the structure of group of cilia (†) appears.

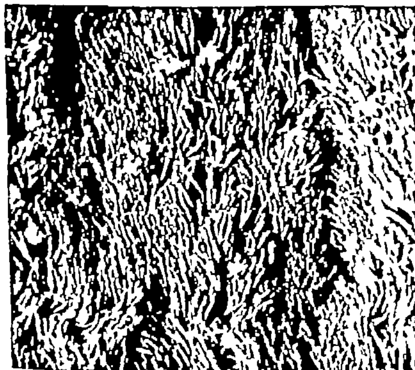


Fig. 2 Normal human inferior turbinate. 3 000. Free surface of the mucous membrane. Groups of cilia wave in a certain direction together with the adjacent groups of cilia.







Fig. 5 Isolated ciliated cell of normal human inferior turbinate. 3 000. View of the ciliated cell. The isolated ciliated cells taper off at the proximal portion and are viewed as being rougher on the outer surface. Numerous cilia ( ) grow up through the free sur ace of the cells.



Fig. 6 Human nasal polyp 10 000. Goblet cells The free surface of the nasal polyp is cov ered by the goblet cells with microvilli. The cell boundaries of groups of microvilli are discriminated. All microvilli are crowded, regularly arranged on the cell surface and ha e a short club-like appearance.

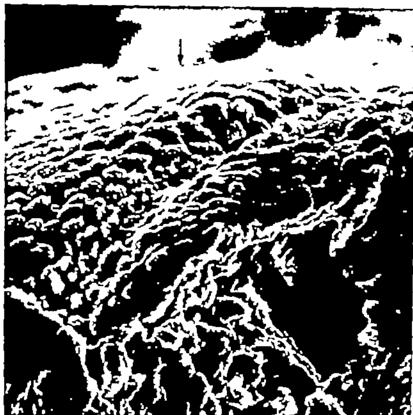


Fig. 7 Involved human inferior turbinate. × 1 000. Lining of squamous cell. Both free (↓) a[?] sectioned surface (←) of the mucous membrane are together visualized. The free surface is covered with cells (→) similar in the appearance to flat squamous cell, and the sectioned surface is packed with ground substance (↑).



Fig 8 Rabbit nasal septum. × 3 000. The basal cells of the lining epithelium. Numerous p gonal or elongated cells appea showing numerous processes li microvilli on the outer surface The intercellular connections a pear to be looser and intercell space wider

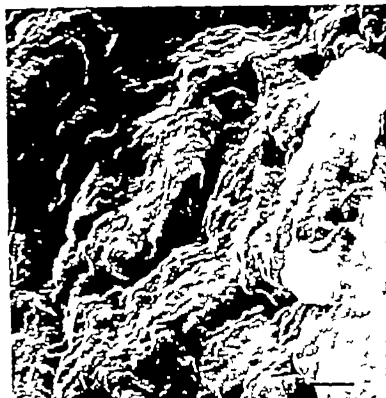


Fig. 9 Human nasal polyp. × 10 000. Sectioned surface of the polyp. The subepithelial layer was packed and covered with homogeneous gelatin-like substance and so the detailed structure of the layer is obscure.



Fig. 10 Rabbit nasal septum. 10 000. Sectioned surface of the mucous membrane. Numerous bundles of fine fibers (↓), being covered partly with the mass of ground substance (↑) are visualized. Some of the bundles reveal regular fine stripes (←) on the outer surface.



Fig. 11. Normal human inferior turbinate. × 3 000. Dense mesh-works of the connective tissue fibers (↑) appear from the mas of ground substance between two venous vessels. The vessels are filled by the red cells (↓) in the lumen, and show a number fine pores on the inner wall (→).

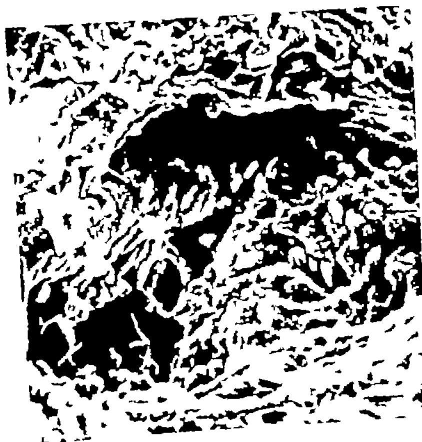


Fig. 12. Human inferior turbinati hypertrophic rhinitis. × 300. The c sectioned lumen is the terminal p of secretory gland. The inner s shows patterns of villus (↑) and s tory granules (←).



Fig. 13 Human inferior turbinate with hypertrophic rhinitis. 1 000 Sectioned surface of the mucous membrane. The structure with lumen is possibly glandular duct. The inner wall shows a spiral pattern (↑) without any structure of lining epithelial cell.



Fig. 14 Human nasal mucous membrane with hypertrophic rhinitis. 3 000. Sectioned surface of the mucous membrane made by the method of paraffin embedding and subsequent usual histologic preparation without staining. Almost all of the mucous blanket and ground substance (↑) disappear. Shrinkage of the red cells (↓) in the vessel and connective tissue fiber (→) are marked

presumed to be metaplasia of the epithelial cell to squamous cell (Fig 7)

5 During scanning of the free epithelial surface of sample 4 numerous polygonal or elongated cells appeared having numerous microvilli-like processes on the outer surface The intercellular connection appeared to be looser and intercellular space wider than in the ciliated cells. There were intercellular processes connecting the adjacent cells with each other These cells must be the basal cells of the lining epithelium (Fig. 8)

6 During scanning of the sectioned surface of the mucous membrane, the subepithelial layer was seen to be packed and covered with a homogeneous gelatin-like substance, thus the detail of structure of the layer was obscure except in the area where the gelatin-like substance was absent. The substance appeared to increase, become swollen and to soften in nasal polyp or involved mucous membrane as compared with the normal one (Fig. 9)

7 Many bundles of fine fibers, being covered partly with the mass of ground substance, were visualized. These varied in size, distribution and running direction. The individual fibril ran and formed bundles, but at the same time separated and branched connecting the adjacent fibril and forming network. The fibril was presumed to be collagen fiber and the branches as the so-called reticulum fiber Some of the bundles revealed regular *fine stripes on the outer surface* (Fig. 10) It was not determined whether the stripes were the same as that of the collagen fiber found in transmission electron microscopy or came from artificial shrinkage and the bundles were really collagen or elastic fiber nerve fiber

8. The venous vessels, differing in size and shape were observed. In the structure of the inner wall the endothelia were not seen but a number of fine pores were distributed uniformly (Fig 11).

9 Structures with lumen were cross-sectioned. One was elongated and its inner surface showed patterns of villus differing in size and form such as elongated, round or oval. The appearance of secretory granules is also visualized on the wall (Fig. 12). Another was irregular in form without any structure of lining cells but showed a spiral pattern on the surface of the inner wall. The former seemed to be sectioned secretory gland and the latter secretory duct (Fig 13)

10. When the specimens were made by paraffin embedding and subsequent routine preparation of the tissue by common histologic technique, artifacts such as shrinkage of the tissue or disappearance of the ground substance and mucous blanket were introduced (Fig. 14)

DISCUSSION

The structures of components in the nasal mucous membrane were visualized as three-dimensional pictures by means of the scanning electron microscope and some new facts were described. The results, however could be predicted from previous description by transmission electron or light microscope.

In preparing specimens, the picture of the mucous layer covering the surface of the membrane is lost in the transmission electron microscope, while in spite of careful washing of the specimens, it remained and disturbed observation in the scanning electron microscope As a result of the difficulty in the removal of the mucous layer in the present experiment, it is known that there are two kinds of mucous, as Proetz (1943) already pointed out. One is thicker and adhered to the tip of the cilia for transporting substances on the mucous blanket and the other is thinner to help cilial movement.

The surface structure of the individual cilia is the same as demonstrated in the transmission electron microscope but the tips of the cilia are duller and less tapered than in previous description (Rhodin, 1959). Furthermore patterns of stripes on the surface of

the cilia can be clearly seen. This pattern is pointed out as an artifact due to shrinkage by Tokunaga et al. (1970) It is characteristic in the present experiment, that the wave-like arrangement of the motile cilia is maintained, as in vivo including metachronal motion of individual cilia and leaning of groups of cilia in a certain direction. The pattern of microvilli on the goblet cells is clear in the present experiment, showing club-like shape and variation of number from cell to cell. The number of goblet cells in the nasal polyp and involved mucous membrane examined increased compared with other specimens.

Beneath the layer of ciliated and goblet cells, an arrangement of polygonal cells with numerous microvilli-like protuberances on the outer surface can be seen. These are the basal cells. From transmission electron microscope pictures, the structure of the basal cell can be imagined (Rhodin, 1959) but in the present paper the surface structure is visualized to understand more easily in desmosome like intercellular connection, the pavement like arrangement, flat polygonal shape and microvilli-like pattern of the outer surface.

Through the transmission electron microscope, the ground substance which fills the intercellular space is seen as a homogeneous electron-dense material with scattered deposits of staining metal in fine granules and can be seen by a special histochemical method for mucoprotein in light microscopy In the present experiment it can be observed as a gelatin-like substance covering the components of the mucous membrane. It is clear which of those is best for real expression. Furthermore, since it appears to be softer and increased in nasal polyps in the present method, a pathophysiological meaning or function of the ground substance can be learned, such as that it increased in involved mucous membrane and absorbed exudate and so on.

A vivid three-dimensional visualization of the connective tissue fibers was also examined in the present method. Numerous fibril-making bundles, or fine meshworks, run in various directions, not only parallel to the basal membrane but vertical or transverse. The fibril-forming meshworks are so-called reticulum fibers and the group of meshworks just beneath the basal membrane are the basement membrane in light microscopy

On the inner surface of the wall of venules, numerous pores can be seen by the present method. As Cauna (1970) described, these venules of the nasal respiratory mucosa are characterized by pores and discontinuities in their endothelial basement membrane, especially designed for the rapid passage of fluid and high molecular solutes through the vascular wall in pictures of the electron microscope of the transmission type.

The structure of the inner lumen of the secretory gland and duct also can be seen in cross-sectioned specimens. The luminal surface of the secretory gland is composed of villus or microvillus, patterned in an irregularly shaped ball-like mass of secretory granula and elongated processes. The luminal surface of the duct as it appeared in the present picture is smooth with a spiral pattern as was presumed. These are the same as seen in transmission electron microscopy

It has been revealed in the present experiment that the structures of components of the nasal mucous membrane can be visualized in three dimension at high magnification, though the observation is limited to the surface However the method still has its problems. During the procedure of preparing specimens, artifacts such as shrinkage can be introduced (Morovitz et al., 1970). Coating by carbon and gold for electric conduction covers the tissue surface and changes them from what they are. Identification of either component of the tissue also awaits further study One difficulty for example, is that the inner structure of the tissue cannot be seen and the histochemical method cannot be applied. In spite of all these unsatisfactory points, the use of the scanning electron microscope will elicit a great deal of information on the struc

ture of tissue apart from that obtained in the transmission electron and light microscope.

ZUSAMMENFASSUNG

Die Strukturen der Nasenschleimhautkomponenten sind mittels des Scanning Elektron Mikroskope untersucht worden. Der die Epithelzellen überziehende Schleim, wie auch die interzellulare Grundsubstanz in der subepithelialen Schicht, ist klar zu sehen gewesen. Folgende Strukturformen konnten in dieser Untersuchung charakterisiert werden, nudelartige Form der Cilia der Flimmerzellen, keulenförmige Fortsätze der Mikrovilli der Becherzellen, vieleckige Form mit zahllosen Mikrovilli der Basalzellen, Bündel und Reticulum-Bildung der Bindegewebsfaser Poren an der inneren Wand der Venüles; Mikrovilli und Sekretionsgranula der inneren Fläche des peripheren Teils der Sekretionsdrüsen, Spiralform der inneren Wand des Glandkanals.

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PSEUDOCROUP WITH STRIDOR

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Abstract. In recent years corticoid therapy has been used in the treatment of pseudocroup (acute subglottic laryngitis) accompanied by respiratory difficulties, with good results. In 103 children (34%) out of a series of 296 with pseudocroup, the condition was accompanied by stridor The effect of corticoid therapy and ACTH therapy on the symptoms was compared. The duration of the stridor after commencement of the treatment was established and recorded. 52 of the children were treated with Solu-Glyc (Erco) and 51 with Synacthen Depot (Ciba). The stridor disappeared after an average of 100 minutes with the hydrocortisone therapy and after 120 minutes with the ACTH therapy

Acute laryngitis in children can be divided into three different types. epiglottitis, pseudocroup or acute subglottic laryngitis, and laryngotracheobronchitis. Epiglottitis is a separate disease, quite different from the others. Acute subglottic laryngitis and laryngotracheobronchitis can sometimes occur together when in general tracheobronchitis is dominant in the disease picture.

Papers describing the symptoms of pseudocroup have been published by Ingelstedt & Tibbling (1966) Kinnman (1970) and others.

Briefly the symptoms are

1 Severe hacking cough.
2 Stridor
3 Anxiety due to a feeling of suffocation.
4 At inspiration the jugulum and epigastrium are drawn in, as is the skin at the intercostal spaces. Expiration is rarely affected.
5 In most cases onset occurs during the night and is associated with an upper airway infection of virus type.

For many years the treatment of pseudocroup has taken the form of nosedrops, cough mixtures, sedatives, and vapour inhalations. Subsequently antibiotics were included, in spite of the viral origin of the infection. Over the years, penicillin has come to be the most common antibiotic used in cases where antibiotic therapy is considered necessary Antibiotics are given to combat any possible secondary bacterial infection. This is all the more justified when corticoids are being given, because of their possible immunosuppressive effect.

Corticoids have been used in the treatment of pseudocroup since the beginning of 1950 and very good results have been reported by Nilsson & Olow (1954), Mårtensson et al. (1960), Leegaard (1960), Ingelstedt & Tibbling (1966) Ross (1969) Kinnman (1970), Andreasson et al. (1971). The dosage has gradually increased from small amounts up to large doses of 100–200 mg sometimes with repeated administration. Corticoids have been administered in the form of a water-soluble preparation by intramuscular injection, which produces a rapid increase in the corticoid concentration in the blood. Recently corticoids in tablet form have also been used in the initial phase of pseudocroup. The advantage of this form of treatment is that it can be carried out even at the patient's home. The results have been reported as good.

ACTH has been tried in the treatment of pseudocroup by Koch (1954) This medication has otherwise mostly been used in laryn-

gotracheobronchitis, but with uncertain results (Turner & Morgan, 1952).

Two cases of pseudocroup accompanied by congenital laryngeal stridor were treated with ACTH in the form of Synacthen Depot (Ciba) during 1971 at the otological clinic in Karlstad. The effect of corticoids in these cases had only been transient. The results obtained with ACTH therapy were very good (Flisberg & Olsholt, 1971). This therapy was subsequently used in several similar cases and even in children with decannulation problems following tracheotomy.

Aim of the investigation

Since ACTH therapy appeared to be effective in pseudocroup it was decided to compare the results of ACTH therapy in the form of Synacthen Depot (Ciba) with those of corticoids in the form of Solu-Glyc (Erco) in patients with pseudocroup accompanied by inspiratory stridor.

Trial preparations

Synacthen tetracosactrin (1–24 corticotrophin) is a synthetic ACTH preparation containing the first 24 amino acids of natural corticotrophin. Adsorption of the active substance to a zinc hydroxyphosphate complex gives the preparation a depot effect (Synacthen Depot) which gives an increased plasma cortisol level 30–48 hours after the injection (Spaethe et al., 1966; Rausch-Stroomann & Petry, 1968, i.a.).

Solu-Glyc is a water-soluble hydrocortisone salt (hydrocortisone sodium succinate) which is quickly broken down into free hydrocortisone in the blood. By both intravenous and intramuscular injection a rapid increase in the blood cortisone level occurs after only a few minutes.

Patient material

296 children with the diagnosis of pseudocroup were treated at the otological clinic, Central Hospital, Karlstad between April 1971 and March 1972 (Table I). From these

Table I. *Children with pseudocroup in-patients, April 1971 to March 1972*

	Total in-patients with pseudocroup	Number of children with pseudocroup and stridor
Boys	209	70
Girls	87	33
Totals	296	103

only those patients with the typical symptoms of pseudocroup including inspiratory stridor were selected for the study. Of the children treated at the hospital, 103 (34%) fulfilled this criterion. The sex and age distribution of these patients are shown in Table II.

As the patients were admitted to the study they were allocated to treatment with Solu-Glyc (100 mg i.m.) or Synacthen Depot (0.5 mg i.m.). Thus, every second patient received Solu-Glyc giving a total of 52 children treated with this drug and 51 children treated with Synacthen Depot. Four of the children in the study with the diagnosis of pseudocroup with inspiratory stridor had already been treated in hospital on several occasions. These children were treated either with Synacthen Depot or Solu-Glyc according to the randomization schedule described above.

Apart from this treatment the patients received routine antihistamine therapy in the form of Lergigan cough mixture with both expectorant and anti-tussic properties, and nose drops (Otrivin Ciba). Children with fever received penicillin in the form of Calcipen-K (Leo) in the appropriate dosage.

Table II. *Sex and age distribution of children with pseudocroup and stridor*

Age (years)	Boys	Girls	Total	%
< 2	29	21	50	49
2–4	19	6	25	4
> 4	22	6	28	27
Totals	70	33	103	100

Table III. *Age and sex distribution of children treated with Synacthen Depot or Solu-Glyc*

	Boys		Girls	
Age (years)	Synacthen Depot	Solu-Glyc	Synacthen Depot	Solu-Glyc
<2	14	15	13	8
2–4	10	9	4	2
>4	6	16	4	2
Totals	30	40	21	12

In order to compare the effect of the two treatments, the time interval between the Synacthen Depot or Solu-Glyc injection and the disappearance of the inspiratory stridor was established and recorded as accurately as possible. The patients were continuously carefully observed.

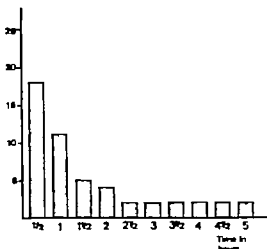


Fig. 1. Duration of stridor in 50 patients after treatment with Synacthen Depot. One tracheotomized patient excluded.

RESULT

Of the total of 296 hospitalized children, 70% were boys and 30% girls (Table I). This proportion is similar to that reported in other investigations.

Of the total number of hospitalized children 103 (34%) had inspiratory stridor requiring treatment with corticoids or ACTH (Table III). Of these, 70 were boys and 33 were girls.

Table II shows that 49% of the children with inspiratory stridor were under 2 years of age, 64% of the girls and 41% of the boys falling in this age group. Approximately 75% of all the patients with inspiratory stridor were under 4 years of age. 32% of the boys and 18% of the girls were aged over 4 years.

It was difficult to establish with accuracy the duration of the inspiratory stridor following the injection of either Synacthen Depot or Solu-Glyc. One has assumed that every form of inspiratory stridor should have disappeared when the point of time was fixed. In Figs. 1 and 2 the duration of stridor is illustrated.

In the patients treated with Synacthen Depot the inspiratory stridor disappeared, on average, 120 minutes after the injection. In the patients treated with Solu-Glyc the inspiratory stridor disappeared on average 100 minutes after the injection. The effect of Solu-Glyc was thus somewhat more rapid than that of Synacthen Depot.

Six tracheotomies were performed during the period of the study i.e. in 2% of the

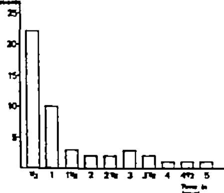


Fig. 2. Duration of stridor in 47 patients after treatment with Solu-Glyc. Five tracheotomized patients excluded.

total number of patients treated for pseudocroup. One of these cases was treated with Synacthen Depot and 5 with Solu-Glyc, in one or more injections. At a later analysis, at least 3 of the tracheotomized children showed symptoms more characteristic of laryngotracheobronchitis than of pseudocroup. All tracheotomized children were considered as being too exhausted to be denied the benefit of tracheotomy The use of drug therapy alone, in the hope of obtaining an improvement was not considered to be justified.

DISCUSSION

In most cases of pseudocroup corticoid therapy has completely changed the course of the disease. The duration of the illness is considerably reduced and this is reflected in a reduction in the mean duration of hospital treatment (Andreasson et al 1971) Furthermore the number of tracheotomies performed has declined remarkably (Lindberg, 1963 Andreasson et al. 1971) In some of these cases however relatively high corticoid dosages are necessary often repeated several times and sometimes for several nights in succession. The risks of short term corticoid therapy are considered to be very small and can be com ly disregarded with the dosages used in oup. With long-acting ACTH preparations, *in the form of Synacthen Depot*, one obtains prolonged stimulation of the adrenals. It should thus be possible to avoid repeated injections.

On the other hand one does not get the same rapid increase in the plasma cortisol level with ACTH preparations as with hydrocortisone. This is probably the reason why the inspiratory stridor disappeared somewhat more slowly with Synacthen Depot than with Solu-Glyc. However the difference between the effects obtained with the two preparations is considered to be of very small practical importance.

In this study rather a high percentage of tracheotomies were performed. However if the three cases with pseudocroup accompanied by signs of laryngotracheobronchitis are excluded the incidence of tracheotomies in all the hospitalized patients is 1%. Even this percentage is high compared with the findings of Andreasson et al. (1971). This may be due, among other factors, to geographical conditions as a result of which these children came to the hospital for treatment at a very late stage. The children were often so ill that it was not possible to await the effect of drug therapy The number of tracheotomized children in the Solu-Glyc group was higher than in the group treated with Synacthen Depot. No conclusions can be drawn from this, since many other factors are also involved, for instance the duration of the symptoms, patient's anxiety and nervousness, infection fever fluid balance etc.

It can generally be said that the effect of ACTH was better than really expected. It is assumed that this was due to the sustained high plasma cortisol level obtained with powerful ACTH depot preparations. The results of the study also suggest that ACTH stimulates the adrenals even in stress situations in children with pseudocroup, where maximum adrenal stimulation is already to be expected.

An old rule says that the symptoms in pseudocroup can be expected to continue for as long after the start of treatment as it was *present before the start of* treatment. This was possibly true before the advent of corticoids but the rule may still be borne in mind and can perhaps serve as a guide in the choice of therapy With a long case history the subglottic oedema is likely to be more marked and for this reason will require a longer period before regression is obtained. In these cases it seems more appropriate to use a preparation which effectively increases the corticoid level in the plasma over a longer period. The effect of hydrocortisone preparations would probably be too brief to exert a favourable influence on the mucous membrane oedema. A long-acting ACTH preparation, however leads to an increased plasma cortisol

level for up to 2 days. In this way it should in many cases be possible to avoid repeated injections.

Encouraged by the good results obtained with Synacthen Depot in pseudocroup, all children with pseudocroup accompanied by inspiratory stridor being treated at the otological clinic, Central Hospital, Karlstad, now receive Synacthen Depot as a matter of routine in addition to the usual basic therapy

ZUSAMMENFASSUNG

Seit mehreren Jahren ist Hydrocortison in der Behandlung der akuten subglottischen Laryngitis mit Atmungsschwierigkeiten benutzt worden. Man hat damit gute Ergebnisse erzeilt. Von insgesamt 296 Kindern mit subglottischer Laryngitis hatten 103 (34%) inspiratorischen Stridor Man hat den Effekt von Hydrocortison und ACTH bei diesen Kindern verglichen. Die Dauer des Stridors nach der Medizinierung wurde gemessen. 52 Kinder bekamen Solu-Glyc (Erco) und 51 Kinder Synacthen Depot (Ciba). Der inspiratorische Stridor ist bei den Patienten die Solu-Glyc bekamen, nach 100 Minuten verschwunden. Bei den Kindern, die Synacthen Depot bekamen, ist der inspiratorische Stridor durchschnittlich nach 120 Minuten erschwunden.

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Acta Otolaryng 76 300–304 1973

ULTRASTRUCTURAL STUDY OF THE HUMAN ANTERIOR PITUITARY

Preliminary Results

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(Received March 14 1973)

During the last decade the fine structure of the hypophysis of many animal species has been studied with the electron microscope But owing to the necessity of immediate fixation, the ultrastructural study of the human pituitary has been limited.

Our study is concerned with the morphological events of hormone secretion and the classification of cell types in the human anterior pituitary As most of our initial investigations have been on pathological material, y tentative conclusions regarding the nor l state can be drawn now A more precise ent of the fine structure of the human y will be obtained when more glands from patients with a variety of pathological conditions have been examined.

Surgical removal of the pituitary gland using the paranasal transethmoido-sphenoidal technique (Escher 1957 1965 1966 1970) allows excellent tissue preservation, since the tissue can be fixed immediately after severing the blood vessels. For the present work human pituitaries were obtained following hypophysectomy for the relief of carcinoma of the breast or of insulin-resistant diabetes, as well as for primitive tumours. The glands were fixed in 4% phosphate-buffered glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in ethanol and embedded in Epon.

In animals it has been shown by autoradiographic studies *in vivo* (Racadot et al 1965) and *in vitro* (Tixier Vidal & Picart 1967), that hormone biosynthesis occurs in the rough endoplasmic reticulum. We found the rough endoplasmic reticulum of human pituitaries to be morphologically similar to that of animal pituitaries. This cellular component is composed of a network of double membranes forming cisternae vesicles and tubules. The outer surface of these membranes are associated with small particles, the ribosomes containing ribonucleoproteins (Fig. 1).

The material synthesized in the rough endoplasmic reticulum appears to move towards the cisternae of the Golgi complex, another membranous system where the formation of secretory granules takes place (Fig. 2). Considerable variation occurs in the shape size and opacity of secretory granules from one cell type to another Such differences have permitted the morphological classification of granulated anterior pituitary cell types (Farquhar, 1966 Bergland & Torack, 1969 Paiz & Hennigar 1970) As indicated in Fig. 3 we

This work was supported by a grant from the Fonds National Suisse de la Recherche Scientifique (No 3.299 70).

The paper was read by F Escher Collegium O.R.L.A.S. Berne Sept. 13 1972.



Fig. 1. Part of a pituitary cell with a well developed rough endoplasmic reticulum (*RER*), ribosomes (*R*), nucleus (*N*).

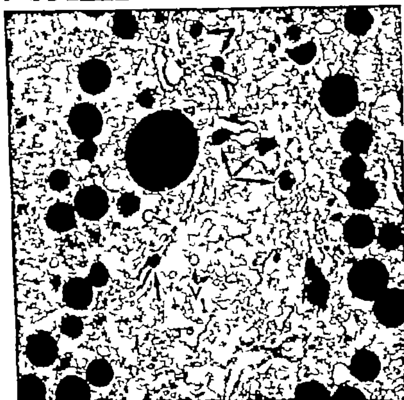


Fig. 2. Golgi complex formed by cisternae (*C*) and vesicles (*V*). The arrows show numerous secretory granules.

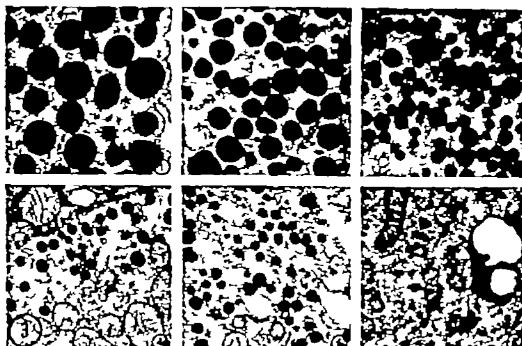
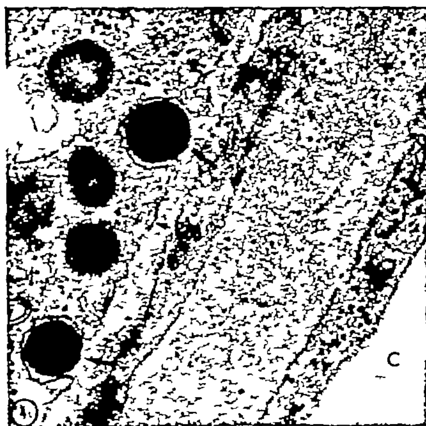


Fig. 3. Six different cell types of the human pituitary can be differentiated by the size and shape of the granules.



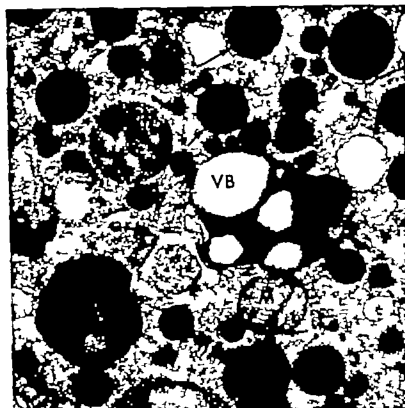


Fig. 5. Besides the secretory granules (↑), a lysosome (*L*) with two granules and a vacuolated body (*VB*) can be seen in this cell. Mitochondria (*M*).

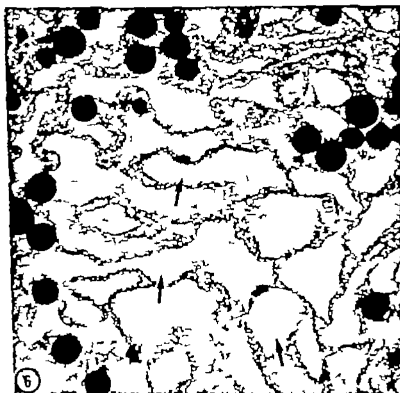


Fig. 6. Pituitary cell in a high secretory state characterized by numerous vesicles (↑) containing pale, homogenous material.

were also able to find at least 6 different granulated cell types in the human pituitary These may be related to the synthesis of six different hormones.

From the Golgi complex, the secretory granules migrate to the cell periphery and are delivered by exocytosis accompanied by fusion of their limiting membrane with the plasma membrane (Fig 4).

Under conditions of reduced demand for hormone product, the cell can achieve the degradation of excess secretory material (Smith & Farquhar 1966) Granule may become incorporated in lysosomes. There they undergo progressive digestion under the action of the hydrolytic enzymes contained in the lysosomes (Fig. 5).

Under conditions of increased demand for hormone product it appears that the condensation of the secretory material by the Golgi complex may be bypassed (Farquhar 1971). The cytoplasm of such a cell is occupied almost entirely by dilated vacuoles containing a flocculent pale material (Fig. 6)

The excellent preservation of our tissue achieved by immediate fixation after surgical removal allows a detailed ultrastructural study of the human pituitary The preliminary re- presented here suggest that the fine tructure of the human anterior pituitary may esemble that of the animal gland with respect to the mechanism of hormone synthesis and release as well as to the variation in number of different cell types. The further comparison of our observations with the fine structure of the animal gland in different experimental conditions will permit a better understanding of the secretory events in the human pituitary and will constitute an approach to elucidate some of the pathological problems in man

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Acta Otolaryng 76 305–312, 1973

TEMPORAL SUMMATION IN THE ACOUSTIC STAPEDIUS REFLEX MECHANISM

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(Received February 12, 1973)

Abstract. Temporal summation in the acoustic stapedius reflex mechanism was studied by monitoring acoustic impedance changes at the ear-drum. The results show that the duration of short acoustic stimuli ("white noise" and 1/1 octave bands of noise) has a pronounced effect on the stapedius reflex response. For a ten-fold increase of stimulus duration the intensity level had to be lowered by about 22 dB in order to elicit the criterion response (50% of maximal obtainable impedance change). For stimulus durations shorter than 50–80 ms, the relationship between sound intensity and stimulus duration was about the same for all types of stimuli, indicating that for such short stimuli temporal summation in the acoustic stapedius reflex is independent of the type of stimulus. For longer durations, several time constants of summation were found. The time constants were largely dependent on the type of stimulus. Possible explanations of these findings are discussed.

Psychoacoustic experiments have established that the loudness of short sounds varies with their duration. Within certain limits the intensity level has to be decreased by roughly 10 dB when the duration of the stimulus is increased by a factor of 10, to maintain constant loudness. This function holds true for sounds with narrow frequency spectra at intensity levels from the hearing threshold to the upper practical limit. A somewhat different relationship exists for broad-band noise at the threshold level (Zwislocki, 1969)

The relationship between loudness and duration of short sounds has been explained as temporal summation in the auditory system. Several experiments have indicated that the locus of temporal summation is in the central nervous system (Zwislocki, 1960, 1969).

The acoustic stapedius reflex in man seems to be loudness-governed (Metz, 1952, Jepsen, 1955 Thomsen 1955 Ewertsen et al., 1958 Klockhoff 1961 Ross, 1968 Flottorp et al., 1971 and others). Reflex contractions of the stapedius muscle are most probably elicited when the neural activity in the superior olivary complex exceeds a certain level. If therefore the temporal summation occurs at or below the level of the superior olive the effect on the acoustic stapedius reflex, measured as changes in the acoustic impedance of the ear should reflect the summation process. Previously the effect of temporal summation on the human stapedius reflex has been demonstrated using a 2 000 Hz tone (Djupesland & Zwislocki, 1971). However tones of short (finite) duration are never pure, because the start as well as the end of the tones causes energy spread to lower and higher frequency bands on both sides of the tone frequency (Fourier 1822). In this respect, octave bands of noise are a more well defined stimulus, the spectrum being less dependent on duration.

The aim of the present investigation was to study temporal summation in the human stapedius reflex mechanism, using "white noise" and 1/1 octave bands of noise as the reflex eliciting stimuli.

MATERIAL AND METHODS

Five subjects with normal hearing 2 female and 3 male (age 17–30 years) took part in the experiments.

The subject was seated with the head fixed in a comfortable position All the measurements were carried out in a sound-proof and anechoic chamber with all measuring and monitoring instrumentation placed outside in the control room.

An earphone (Telephonix TDH 39 Grason Stadler Cushion 00) was placed on the left ear of the subject. The right ear canal was connected to an apparatus designed for registration of sound pressure changes of a 550 Hz test tone due to impedance changes elicited by stapedius muscle contractions. A block diagram of the apparatus is shown in Fig. 1

Reflex contraction of the stapedius muscle was elicited by acoustic stimulation of the left ear by means of bursts of 1/1 octave bands of noise and "white noise" limited by the frequency response of the earphone The centre frequencies of the noise bands were 250 500, 1 000 2 000 and 4 000 Hz (preferred frequencies).

The actual frequency ranges of the stimuli ere controlled by measurements on a 6 cm³ upler the results are presented in Table I.

The time interval between the stimuli was 4 s, and the duration of the noise bursts was varied from 5 to 3 000 ms. The rise and the decay time of the stimuli were 25 ms each. This was achieved by means of a timer (Advance PG 52 A) and an electronic switch (Grason-Stadler Modell 829 C) Correct regulation of the electronic switch and timer was obtained by substituting the noise by pure tones and registering the tone pulses on a storage oscilloscope (Tektronix Type 564 B). The stimulus durations were measured between the half-power points.

During the experiments the sequence of the stimulus durations used was as follows. 5 20, 50, 200, 500 and 3 000 ms, followed by 10, 30 100, 300 and 1 000 ms. This procedure was chosen to prevent the results being biased by a fixed increase in stimulus duration.

A soft rubber plug with two polyethylene tubes, 11 cm long, was placed in the right ear canal of the subject, making an airtight fitting close to the tympanic membrane. One of the polyethylene tubes was connected to a Brüel & Kjær condenser microphone (Type 4134) serving as transducer for the test tone generator introducing a 550 Hz tone in the cavity The other polyethylene tube was connected to another Brüel & Kjær microphone (Type 4134) in order to register sound pres-

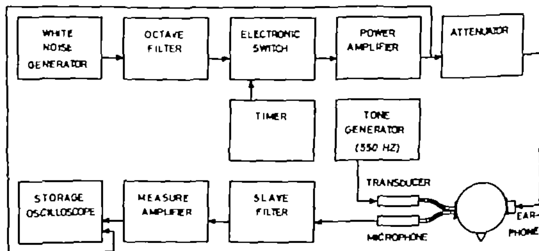


Fig. 1 Block diagram of the apparatus used for eliciting and recording the acoustic stapedius reflex.

Table I *Frequency range of the stimuli used measured in a 6 cm³ coupler*

The limiting frequency is defined as the frequency at which the spectrum level is reduced 10 dB

Octave (Hz)	Lower limiting frequency (Hz)	Higher limiting frequency (Hz)
250	150	400
500	310	850
1 000	600	1 700
2 000	1 300	3 300
4 000	2 400	6 300
"White noise"	45	8 300

sure level. The signal from the microphone passed a Brüel & Kjær Measuring Amplifier (Type 2606) and a Brüel & Kjær Slave Filter (Type 2020) and was finally recorded on a storage oscilloscope (Tektronix, Type 564 B). The sound registering microphone was calibrated by means of a Brüel & Kjær 2 cm³ calibration coupler Type DB 0260. The intensity of the test tone, measured in the small cavity between the rubber plug and the tympanic membrane was 80 dB SPL, tested to be below the acoustic stapedius reflex threshold.

The intensity of the noise bursts was varied in 1 dB steps until the resulting stapedius reflex response in the contralateral ear resulted in an 1.4 dB increase in test tone intensity—representing 50% of the maximal obtainable impedance change ("criterion response"). A graphical interpretation of the relationship between stimulus intensity and size of impedance change elicited by bursts of "white noise" with duration 50 and 500 ms, showed an S-shaped curve, the steepest part of the curve being about 50% of maximal obtainable impedance change, corresponding to 1.4 dB increase in test tone intensity. In the present investigation, therefore, 50% of the maximal obtainable impedance change was chosen as the criterion response.

The time constants of the apparatus were found to be such that they did not interfere with our measurements (Sundby et al. 1971).

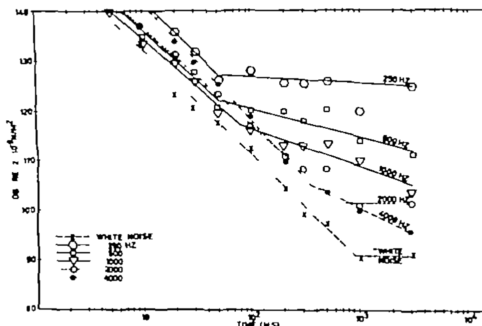


Fig. 2. Relationship between sound pressure level and stimulus duration required to elicit the criterion response (50 % of maximal obtainable impedance change) for the different types of stimuli used. Mean value of three measurements on one subject.

MATERIAL AND METHODS

Five subjects with normal hearing 2 female and 3 male (age 17–30 years) took part in the experiments.

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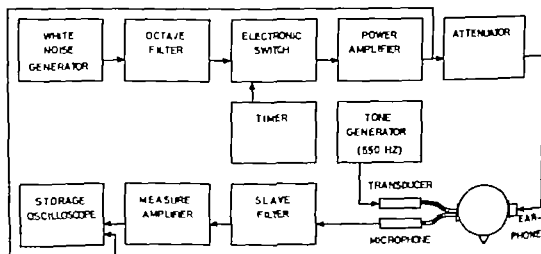


Fig. 1 Block diagram of the apparatus used for eliciting and recording the acoustic stapedius reflex.

Table II. *Median values and interquartile ranges (I Q.R.) for stimulus durations and intensities at "knee-points" together with the slope of the curves on both sides of the "knee-points"*

The numbers referring to the slope (below and above "knee-point") indicate how many dB the stimulus intensity level had to be lowered for a ten-fold increase of duration in order to elicit the criterion response

Stimulus		Slope below "knee-point"	"Knee-point" time (ms)	Knee-point" level (dB)	Slope above "knee-point
"White noise"	Median	21.5	510	101	6.3
	I Q.R.	4.2	165	11	4.4
250	Median	21.9	80	123	2.1
	I.Q R.	2.1	18	5	0.4
500	Median	25.5	50	119	4.4
	I.Q.R.	2.7	6	9	0.8
1 000	Median	20.4	75	113	7.6
	I.Q.R.	2.7	26	8	0.8
2 000	Median	23.4	575	95	0
	I.Q.R.	4.2	45	4	0
4 000	Median	19.8	600	101	9.3
	I Q.R.	5.9	700	8	6.3

ried out on 2 of the 5 subjects, using "white noise" as *stimulus* and 25% *of maximal obtainable* impedance change as criterion response. Irrespective of the magnitude of the criterion response, the "knee-point" time and the slope of the curves on both sides of the "knee-point" were about the same. In addition the time delay between the stimulus and the resulting change was observed As shown in Fig. 4 the time delay was about 50–100 ms.

DISCUSSION

The results show that the duration of short acoustic stimuli has a pronounced effect on the stapedius reflex response. The effect may be explained as temporal summation in the acoustic stapedius reflex mechanism. Our findings indicate that several time constants of summation are reflected in the acoustic stapedius reflex mechanism and that these are largely dependent on the type of stimulus. For *stimulus durations shorter than the* "knee-point" values (50–80 ms) the slope of the curves was about the same for all types of stimuli used. This indicates that for such short stimuli the temporal summation is in*dependent of the type of stimulus.*

The median slope for stimulus duration shorter than the "knee-point" value (see Fig. 3) shows that for a ten-fold increase of duration, the intensity level to be lowered by about 22 dB in order to elicit the criterion response. This agrees well with previous observations made by Djupesland & Zwislocki (1971) Using a 2 000 Hz tone they found a ten-fold increase of duration to be compensated by about 25 dB decrease of the intensity level of the tone bursts.

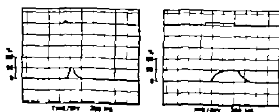


Fig 4 Oscilloscope picture (drawing) showing the time delay between the stimulus (upper trace) and the resulting impedance change (per cent of maximal obtainable change) measured in the contralatral ear (lower trace). Stimuli: Bursts of "white noise" of duration 50 ms (left drawing) and 500 ms (right drawing).

Our results demonstrate a definite difference in the "knee-point" time for frequencies below and above 1 000 Hz. A possible explanation may be a change in stimulation of the inner ear (the "acting intensity") below and above this frequency caused by the middle ear muscle reflex itself. It is known that the reflex contraction of the stapedius muscle produces a sound transmission loss to the middle ear. This loss is most pronounced in the low frequency range (Borg, 1968).

The latency of the acoustic stapedius reflex (time interval between onset of stimulus and recorded impedance change) has been reported to be between 25–150 ms (Metz, 1951 Möller 1958 Terkildsen, 1960). Our findings are in agreement with this (Fig. 4). As a consequence acoustic stimuli of shorter durations than 25–150 ms should show no change in "acting intensity" caused by the middle ear muscle reflex. For longer durations an effect on the "acting intensity" may be expected. Borg (1968) has studied the effect of the human acoustic stapedius reflex on sound transmission through the middle ear and reported that at higher frequencies (2 000–3 000 Hz) the attenuation was very slight. However at low frequencies an attenuation up to 12–15 dB was observed.

Our findings, which disclosed a shorter "knee-point" time for low than for high frequency sounds, may correspond to the effect of the transmission loss observed by Borg (1968). Due to the transmission loss in the low frequency range, higher intensities are required to elicit the criterion response, when low frequency stimuli of a longer duration are used. The actual time (50–80 ms) lies within the range for which sound transmission changes may be expected. Comparing low and high frequency 1/1 octave bands of noise with stimulus duration 1 000 ms, the difference in intensity needed to produce the criterion response was found to be 10–20 dB (Fig. 3).

White noise contains both high and low frequencies. The acoustic stapedius reflex mechanism seems to be dominated by the high frequency component in such a complex sound, because no transmission loss occurs in this frequency range (contrary to the low frequency range). The loss of energy in the low frequency range due to middle-ear transmission reduction is probably more than compensated for by the fact that the energy increases with 3 dB per octave towards higher frequencies. In addition the loudness of the "white noise" is increased because the critical band width is surpassed.

The genuine temporal summation effect is therefore demonstrated either for stimuli with frequencies above 1 000 Hz, or for stimuli below this frequency provided the stimulus duration is less than 50–80 ms. For these two types of stimuli no transmission loss to the inner ear occurs. Whether the transmission loss in the low frequency range alone is responsible for the difference in the "knee-point" values, cannot be deduced from our present data. A possible way to investigate this effect is to get hold of subjects with normal hearing and unilateral paralysis of the stapedius muscle (patients with Bell's palsy and paralysis of the stapedius muscle). We hope to be able to report on such a study very soon.

The stapedius muscle itself and the efferent part of the acoustic stapedius reflex arc are included in our measurements of temporal summation. In the present study we have, however only compared temporal summation resulting from different stimuli. Thus we have not considered variations due to the stapedius muscle itself nor the efferent part of the reflex arc and the transmission between the 8th and the 7th cranial nerves.

Since temporal summation has been demonstrated in the acoustic stapedius reflex mechanism at least some of the summation must take place at or below the superior olivary complex (Djupesland & Zwislocki, 1971). It is, however possible that further summation may take place at higher levels, resulting in different time constants when measured by

psychoacoustic methods. Results from psychoacoustic measurements in fact demonstrate a less steep slope of the intensity/duration function loudness level increases roughly 10 dB when the duration is increased by a factor of 10 (Zwislocki, 1969)

The size of the critical band-width measured by means of the acoustic stapedius reflex and by psycho-acoustic methods (loudness comparison) is not the same (Flottorp et al., 1971 Djupesland & Zwislocki, 1972) It seems very likely that in the temporal summation mechanism similar differences may appear when different methods are employed, one which involves the hearing pathways to the level of the superior olive the other to higher cortical level.

A clear frequency dependence in temporal summation appears not only from our investigation employing an objective registration method, but also from several psycho-acoustic experiments (Chamberlain & Zwislocki, 1970· Gengel & Watson, 1971 Pedersen & Elberling, 1972)

The continued summation effect beyond the "knee-point" (Figs. 2 and 3) is difficult to explain. This effect may however be typical for the level up to the superior olivary complex, and may be compensated for at higher levels. Functionally it is interesting to compare it with adaptation phenomena, which may occur at higher levels and be counteracted by the observed continued summation (increase) thereby keeping the loudness level constant.

The slope of the curves for shorter durations than the knee-point" show little variation with frequency or with subject. The location of the knee-point" and the slope of the curves for longer durations (Table I) showed larger inter-subject variations. This seems quite natural, since at least part of the reason for these variations is to be found in different transmission loss through the middle ear caused by the stapedius reflex. Inter-subject variations have been demonstrated regarding this effect (Borg 1968). It also fits in very well with the observed variation in loudness determination of short sounds reported from the international Round Robin Test, carried out by ISO TC 43 Acoustics (Pedersen, 1972).

ZUSAMMENFASSUNG

Zeitliche Summation in dem akustischen Stapedius-Reflexmechanismus wurde durch die Änderungen der akustischen Impedanz des Trommelfells studiert. Die Ergebnisse zeigen, dass die Dauer kurzer akustischer Reize („weisses Rauschen und 1/1 oktavbandiges Rauschen) einen ausgesprochenen Effekt auf die Reflexantwort des Stapedius hat. Eine 10-fache Vergrösserung der Darbietungszeit erfordert, dass der Schallpegel um 22 dB verkleinert werden muss, um immer die „kritische Antwort hervorzurufen (50% der maximal erreichbaren Impedanzveränderung). Für eine Impulsdauer kürzer als 50–80 ms war das Verhältnis zwischen Schallpegel und Darbietungszeit ungefähr das gleiche für alle Schallreize. Man kan daraus entnehmen, dass die zeitliche Summation im akustischen Stapedius-Reflex für solche kurze Darbietungszeiten von der Art des Schallreizes unabhängig ist. Für längere Darbietungszeiten wurden mehrere verschiedene Zeitparameter der Summation gefunden. Die Zeitparameter waren in der Hauptsache von der Art des Schallreizes abhängig. Mögliche Erklärungen dieser Befunde werden erörtert.

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Acta Otolaryng 76: 313–318, 1973

THE PASSAGE OF INTRACISTERNALLY INJECTED ALBUMIN AND UREA INTO PERILYMPH

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(Received February 19 1973)

Abstract. The total protein and urea concentrations in perilymph, CSF and serum were determined following intracisternal injection of albumin and urea in cats. Total protein concentration in perilymph increased abruptly when total protein in CSF increased following injection of albumin. The total protein concentration in perilymph equalled two-thirds of the protein concentration in CSF by the first hour and remained fairly constant throughout the second and third hours although the protein concentration in CSF decreased during this time. The concentration of urea in both perilymph and serum increased as urea concentration in CSF increased. However urea concentration in perilymph returned to the original value after 2 hours. This concentration remained constant and paralleled serum urea concentration even though the level of urea in CSF remained approximately five times higher than its initial value. The relationship between these findings and the etiological factors of clinical disorders such as antibiotic toxicity Menière's disease and hearing impairment after hemodialysis are discussed.

The pathological modification of biochemical mechanisms regulating the inner ear fluids formation and circulation is often speculated to be a basic cause of Menière's disease.

It has been suggested that perilymph may be derived from the following sources: 1) capillary walls of perilymphatic space, 2) fluid under the nerve sheaths and 3) the cerebrospinal fluid (CSF) (Palva & Raunio, 1967 1968). However the degree of the participation of these factors in the formation of perilymph has not been thoroughly studied.

It is generally assumed that there is an open communication through cochlear aqueduct between the perilymphatic space of the cochlea and the subarachnoid space of the brain (Anson et al., 1964 Anson 1965 Palva & Dammert, 1969). This assumption has been based on the histological observation of the distribution of intracisternally injected materials in the inner ear. Many investigators have reported that when fluorescein or dye solution was injected intracisternally this foreign substance appeared in the scala tympani within a short period of time (Cisselson, 1949; Jako et al., 1959). However there has been little information available on the process of distribution into CSF perilymph and serum of intracisternally injected substances.

This study was designed to follow the time course of the changes in concentration of intracisternally injected substances in the CSF perilymph and serum in order to provide basic information regarding the effect of CSF composition on the composition of perilymph and serum. Two substances with different molecular weights (albumin, 69 000; urea, 60) were used as test materials, and the distribu-

This research was supported by a grant from USPHS (NS 03349) and a Graduate Research Grant from the University of Minnesota, Minneapolis, USA.

tion patterns of these substances were determined in CSF perilymph and serum after intracisternal injections.

MATERIAL AND METHOD

Cats ranging in weight between 2.5–3.0 kg were used. All cats were anesthetized by intraperitoneal injection of Nembutal (30 mg/kg).

Blood, CSF and perilymph were collected before and 1 2, and 3 hours after the injection of albumin (50 mg/kg) or urea (10 mg/kg) into the cerebrospinal space through the membrana atlanto-occipitali.

The blood samples were taken from jugular veins. Cerebrospinal fluid was collected from the membrana atlanto-occipitali. Perilymph was obtained from the scala tympani by inserting a glass capillary tube through the round window membrane.

Through a pilot study we confirmed the flow of radioactive albumin (^{125}I-albumin) from CSF into the perilymph by counting the radioactivity in perilymph after intracisternal injection of radioactive albumin.

Since the volume of perilymph is small (around 10 μl) total protein was determined ...d of albumin by a spectrophotometric ...od (Waddell, 1965 Murphy & Kiess, 1960). After diluting the samples (serum CSF and perilymph) with 0.1 M phosphate buffer (pH 7.2) the absorbance of these solutions was measured at 215 and 225 nm. Protein concentrations were obtained from a standard curve which was made using 20–100 μg/ml concentration of Versatol (Warner-Chilcott Lab New Jersey).

Urea nitrogen was determined by a colorimetric method utilizing urease and the Berthelot reaction (Faucett & Scott, 1960 ... ney & Marbach, 1962). A commerci... able test kit (Boehringer ... TC URI 15954) was used.

The following solutions were used. ... urease/ml 2) 0.03 mg urea/ml, 3) 0... phenol, 0.00017 M sod... 0.11 M sodium hypochlorite 0.125 M sodium hydroxide.

Ten microliters of urease solution was pipetted into a plastic cup and 2 μl of samples were added. Only urease solution was added to the blank cup. To another cup, 20 μl of urea standard and urease were added. These were incubated in a water bath at 37 C for 15 minutes. At the end of this time the cups were removed from the water bath and 0.5 ml of solution 3 and 0.5 ml of solution 4 were added to each cup. The cups were again covered and placed in the water bath and incubated at 37 C for 30 minutes. Following the incubation they were transferred into the matching quartz microcuvettes (1.0 cm light path). The optical density of the standard and sample were read against a blank at 540 nm using a Zeiss PMQ II spectrophotometer.

RESULTS

In the study with albumin there was a marked increase relative to the normal condition, in total protein in CSF (20 times) and in perilymph (2 times) 1 hour after the intracisternal injection of albumin. There was a considerable decrease of total protein in CSF by 2 or 3 hours, but no change in total protein concentration in perilymph. No change in total protein concentration in serum was detected at any time (Table I Fig. 1).

In the urea study there was a marked increase of urea in CSF (7 times) and in perilymph (2 times) and slight increase in urea concentration in serum (1.4 times) 1 hour after intracisternal injection of urea. Two hours after the injection the urea concentration in perilymph and serum had returned to their initial values although concentration of urea in CS... ...d about 5 times its initial value (T... 2).

... ...hlear aque... ...al fluid

Table I *Protein concentration in cat serum CSF and perilymph at various times after intracisternal injections of albumin (50 mg/kg body weight)*

	Serum (mg %)	CSF (mg %)	Perilymph (mg %)
Normal values	5 950 ± 150[a] (12)[b]	32.2 ± 1.3 (9)	209 ± 16 (7)
One hour after injection	6 060 ± 160 (9)	625 ± 110 (7)	450 ± 67 (3)
Two hours after injection	5 910 ± 220 (7)	395 ± 71 (5)	430 ± 19 (4)
Three hours after injection	5 470 ± 115 (7)	360 ± 55 (6)	425 ± 24 (3)

[a] Standard error of the mean.
[b] Numbers in parentheses indicate the numbers of samples.

flows from the subarachnoid space to the scala tympani of the cochlea. If perilymph is derived from cerebrospinal fluid and is continually being absorbed and being replaced by a flow through the aqueduct, then occlusion of this pathway should lead to functional disturbance of the inner ear. Uyama (1933) produced a block of cochlear aqueduct of rabbits by inserting a fine thread of catgut into the lumen. He reported that Reissner's membrane was bulged into the scala vestibuli in varying degrees, depending on the efficiency of the blocking and its duration.

Silverstein et al. (1969) reported that perilymph total protein concentration was elevated 2–3 times compared with control values 5 days following the surgical obstruction of the cochlear aqueduct. The elevation of total protein concentration in perilymph remained for about 4 months after the blockage of the cochlear aqueduct. They did not observe any changes in sodium, potassium, and glucose concentrations in perilymph. They speculated that CSF flows through the cochlear aqueduct, after which water is probably absorbed into the veins of the perilymphatic space. They also speculated that a continuous flow of CSF through the cochlear aqueduct may help keep the perilymph from accumulating metabolic waste products and contaminants.

In the albumin study it was shown that perilymph total protein concentration increased abruptly when total protein in CSF increased. However, total protein concentration in perilymph reached two thirds of the protein concentration in the CSF by the first hour and remained fairly constant through the second and third hours while protein concentration in CSF decreased. Thus an increase in perilymph protein follows CSF protein increase suggesting a flow of CSF into the scala tympani. However, the maintenance of a con-

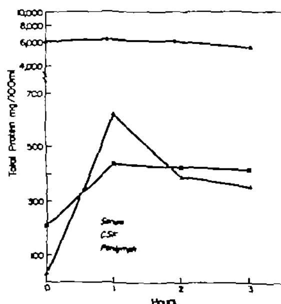


Fig. 1 Total protein concentration in cat serum, cerebrospinal fluid (CSF) and perilymph at various time points after intracisternal injection of albumin (50 mg/kg body weight).

Table II *Urea nitrogen concentration in cat serum CSF and perilymph at various times after intracisternal injection of urea (10 mg/kg body weight)*

	Serum (mg %)	CSF (mg %)	Perilymph (mg %)
Normal values	23 7±1.3[a] (14)[b]	15 7±0.9 (14)	22.6±2.8 (8)
One hour after injection	33 6±4.3 (8)	109.0±6.6 (7)	42.3±5.3 (8)
Two hours after injection	24.2±2.2 (9)	74.9±9.5 (8)	26.8±2.0 (8)
Three hours after injection	24 1±1 1 (14)	51.6±3 4 (11)	28.7±3.9 (3)

[a] Standard error of the mean.
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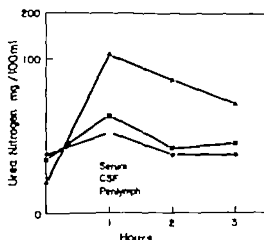


Fig 2 Urea nitrogen concentration in cat serum, cerebrospinal fluid (CSF) and perilymph at various time point after intracisternal injection of urea (10 mg/kg body weight).

Clinically it is known that CSF gamma globulin and total protein increase occur in about 80% of patients with multiple sclerosis, neurosyphilis or subacute leukoencephalitis and in some cases of myxedema. It is interesting to note that congenital or acquired syphilis (7%) and myxedema (3%) have been identified as two specific etiologies of Menière's disease (Pulec, 1972) Although we do not have total protein data on CSF and perilymph of these patients, perhaps the increase of protein in perilymph might have taken place in these patients.

In the urea study the concentration of urea both in perilymph and in serum increased as urea concentration in CSF increased. However urea concentration in perilymph returned to its original values by 2 hours and remained constant and paralleled serum concentration even though the concentration of urea in CSF remained approximately five times higher than its original value. This may indicate that there is a rapid turnover or equilibrium of urea between blood and perilymph which probably involves the perilymphatic vessels. It has been shown that urea diffuses freely through most cellular membranes and any barrier which might exist between perilymphatic space and blood seems to be readily permeable to urea (Youngs & Juhn 1972).

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hemodialysis combined with the use of the anti-heparinizing agent (Ransome et al. 1966). Certain neurological manifestations (convulsion, headache, nausea, vomiting) after hemodialysis have also been reported (Tyler 1965). The cause of this dysfunction seems to be at least in part related to rapid efficient dialysis. In hemodialysis, acute decline in the level of blood urea occurs and because of the slow transport of urea across the "blood–brain barrier" an osmotic gradient is produced in which water moves from the blood and extracellular fluid into the central nervous system. On the other hand, Arslan (1969) has demonstrated vestibular functional alterations by the artificial variation of the osmotic pressure of the perilymph in cat, and he suggested that Menière's attack may be due to a loss of biochemical and osmotic balance of the inner ear fluids when the permeability of the labyrinthine vessels is altered.

The present study demonstrates the different pattern of distribution of chemical substances of different molecular weight in perilymph when they are injected into the cerebrospinal space. It is interesting to observe the slow excretion of total protein from perilymph. This may have some correlation to the functional disturbances of the inner ear when conditions of elevated protein concentration in the perilymph are induced. The changes observed in the difference between CSF and perilymph in urea concentration during the time course of our experiments implies that a corresponding change in osmotic gradient was occurring. Further studies are necessary to determine whether these changes in osmotic gradient can cause the morphological alterations of the membranous labyrinth and functional disturbances of auditory and vestibular organs.

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Nach intrazisternalen Injektion von Albumin und Harnstoff wurde die Konzentration von Gesamteiweiss Harnstoff in der Perilymphe, im Liquor cerebrospinalis und Serum von Katzen bestimmt. Nach Injektion von Albumin erfolgte ein steiler Anstieg der Gesamteiweisskonzentration in der Perilymphe der sich zunächst parallel zu dem Anstieg der Gesamteiweisskonzentration im Liquor cerebrospinalis verhielt. Der Totalanstieg der Gesamteiweisskonzentration in der Perilymphe hingegen betrug nur Zweidrittel des Anstiegs im Liquor, verlief aber weiterhin unverändert während der folgenden zwei Stunden, obwohl in der Gesamteiweisskonzentration des Liquors ein signifikanter Abfall zu verzeichnen war. Nach anfänglichem Anstieg fiel die Harnstoffkonzentration in der Perilymphe nach zwei Stunden auf den Ausgangswert ab und entsprach der Serumkonzentration, obwohl sich im Liquor ein fünffacher Wert der Ausgangskonzentration verzeichnen liess. Ausgehend von diesen Ergebnissen werden im besonderen die Faktoren diskutiert, die in der Etiologie klinischer Syndrome wie z.B. Antibiotikatoxizität, Ménièresche Erkrankung und Hörverlust nach Hemodialyse eine wichtige Rolle zu spielen scheinen.

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Table II *Urea nitrogen concentration in cat serum, CSF and perilymph at various times after intracisternal injection of urea (10 mg/kg body weight)*

	Serum (mg %)	CSF (mg %)	Perilymph (mg %)
Normal values	23 7±1.3 (14)[b]	15 7±0.9 (14)	22.6±_.8 (8)
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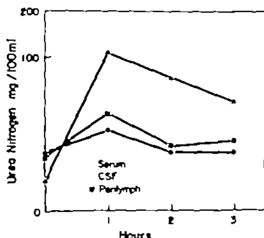


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Table I Serum CSF and cochlear fluid alkaline and acid phosphatase activity in normal and post mortem fluids (µmol/min/l)

	Alkaline phos-phatase	Acid phos-phatase	Inhibition (%)	
			Tartrate	Formal-dehyde
Normal fluids				
Serum (N = 21)				
Mean	46.7	5.4	30	90
S.D	10.9	1 7	11	11
Range	25–65	2–8	5–52	69 100
CSF (N = 1?)	trace	trace		
Post-mortem fluids				
Serum (N = 29)				
Mean	482	204	36	86
S.D	602	?49	19	13
Range	36–2 556	11–540	1–80	48–100
CSF (N = 30)				
Mean	31	23	28	88
S.D	53	13	21	14
Range	0–290	3–46	0–90	61–100
Perilymph (N = 28)				
Mean	9.2	12.2	35	75
S.D	13 4	9 0	31	28
Range	0–53	3–39	0–100	0–100
Endolymph (N = 3)				
Mean	8	11.5	44	80
S.D	7 7	3 7		
Range	. 17	7–14		

1969). Normal sera and CSF samples, avail- in the laboratory were analysed for nor -e data. If the measurements were not ormed the same day all specimens were deep-frozen and kept at −70 C until the time for analysis.

The detailed technique of quantitative determination of alkaline and acid phosphatase activity as well as the technique for electrophoretic separation of isoenzymes is given in another publication (Palva et al. 1973)

RESULTS

The average values of quantitative analyses of alkaline and acid phosphatase activity and the inhibition percentages by tartrate and formaldehyde on acid phosphatase activity in different fluids are shown in Table I For perilymph analyses, samples of two to four ears had to be pooled in order to obtain a large enough specimen. As to endolymph, pooling of all specimens from ten or more temporal bones was necessary to obtain sufficiently large samples for quantitation. Even then it was possible to make only three quantitative analyses on the samples obtained from the 64 temporal bones from which both cochlear and utricular endolymph had been collected.

The average post-mortem serum activity values of both alkaline and acid phosphatase were 10 respectively 40 times, higher than those obtained in healthy persons during life in this laboratory In CSF our average normal figures showed only a trace of activity for both enzymes, whereas a distinct activity was demonstrated post mortem. However the figures remained in the range of 1/10 of the corresponding values in serum. The average alkaline and acid phosphatase activity in perilymph and endolymph was nearly the same for both, representing 1/3 to 1/2 of the post mortem CSF activity

The inhibition tests with tartrate and formaldehyde showed similar results in all test fluids. Thus, most of the acid phosphatase was tartrate-stable and formaldehyde-labile.

The electrophoretic separation of alkaline and acid phosphatase isoenzymes on Difco Special Agar showed no bands initially Later using post mortem sera with known high activity and concentrated samples of CSF and perilymph, weak bands appeared for both enzymes. Alkaline phosphatase activity appeared in the form of one weak band migrating anodically and having a mobility of α_2 globulins. Acid phosphatase activity also appeared (at pH 6.2) as one weak band migrating cathodically with the mobility of β_1-globulins. Using various concentrations these bands were stronger or weaker but no additional bands were brought into view

COMMENT

Earlier data on alkaline and acid phosphatase activity in cochlear fluids or CSF are very

scarce indeed. Rauch (1964) reported that alkaline phosphatase activity was 2.6 Bodansky units in perilymph obtained during surgery for otosclerosis, while in the corresponding sera the average figure was 1.8. In international units these figures would correspond to values about 15 and 10 μmol/min/l which are both lower than our lowest normal serum values. In Rauch's study one patient without otosclerosis showed no alkaline phosphatase activity in the perilymph. There were no data for endolymph and CSF. Our normal CSF analyses showed that this fluid contains only a trace of alkaline and acid phosphatase activity. The blood-CSF barrier apparently is sufficiently effective to prevent filtration of these enzymes into CSF space. The fact that the two perilymph samples of Rauch showed distinct alkaline phosphatase activity in otosclerosis may be due to the active bone process, because the otosclerotic bone surfaces are often in immediate contact with the inner ear spaces.

Our post mortem figures indicating highly increased serum alkaline phosphatase activity reflect the extent of the liver damage during the terminal stages of the disease. No bone tumours or even bone metastases were found at autopsies and none of the ears were affected with otosclerosis. The increased serum acid phosphatase activity post mortem is apparently due to the fact that after death this enzyme is liberated from the hemolysing red cells, as indicated also by the results of the inhibition tests. The high figures in serum were in no way reflected in the activity values of CSF.

In seven post-mortem ears Rauch found perilymph alkaline phosphatase activity of 0.4 Bodansky units (2.1 I.U.) while five specimens of endolymph showed no activity. In three post mortem samples acid phosphatase perilymph activity was reported to be 1.97 King units, and endolymph 0.34 King units in two specimens (3.4 and 0.5 I.U. respectively).

Our figures for alkaline and acid phosphatase activity in post-mortem perilymph and endolymph are clearly larger than Rauch's, but applies to all our results even normative data. The present cochlear fluid activity figures are lower than the corresponding figures in CSF though, because of the wide range of results, the statistical significance is marginal. Both cochlear fluid and CSF values are significantly ($p<0.01$) smaller than those for serum and the bulk of the post-mortem enzyme activity must be interpreted as arising from the capillaries surrounding these fluid spaces. Acid phosphatase activity obviously derives from the hemolysing red cells, most of the enzyme consisting of the formaldehyde-labile erythrocyte type. Alkaline phosphatase may be liberated from the capillary walls and partly also originate from the serum through capillary leakage.

During life, the blood–inner ear barrier apparently acts in the same way as the blood–CSF barrier in the case of both alkaline and acid phosphatase. The molecular weights of most of the enzyme fractions are in the order of 100 000 or more, and it is therefore understandable that filtration from the blood into the CSF or inner ear fluids does not occur during life. The smallest fraction of acid phosphatase, a tartrate-stable fraction having a molecular weight around 35 000 (Georgatsos, 1965) might have an easier passage through the capillary walls during life also. However quantitatively speaking, the figures would probably remain very small, in the class of trace activity similar to CSF.

There seems to be clear evidence for the fact that, as also regards alkaline and acid phosphatase activity, CSF and cochlear fluids have a similar composition and, though the isoenzyme pattern is the same, both clearly differ quantitatively from serum. This adds some weight to our previous conclusion that in normal conditions the protein composition of cochlear fluids is largely dependent upon CSF: the dimensions of the cochlear aqueduct (Palva & Dammert, 1969) permit a free transport back and forth of even the largest protein molecules while filtration could not occ-

cur easily through the intact capillary barriers.

ZUSAMMENFASSUNG

Die Aktivität der alkalischen und sauren Phosphatasen war im post mortalen Serum 10 bzw 40 mal grösser als in normalem Serum. In normaler Zerebrospinalflüssigkeit gab es nur eine minimale Aktivität, während die postmortalen Werte 1/10 den entsprechenden Serumwerte ausmachten. Peri- und Endolymphe hatten dieselbe Aktivität, beide 1/3 bis 1/2 der postmortalen Aktivität in der Zerebrospinalflüssigkeit. Nur ein Isoenzymband wurde für beide Enzym vorgelegt, für alkalische Phosphatasen mit α_2-Globulin und für saure Phosphatasen (pH 6,2) mit β_2-Globulin-Mobilität. Die Aktivität der sauren Phosphatasen war von derselben Art wie in Erythrozyten. Die Ergebnisse zeigen, dass die quantitative Aktivität in der Perilymphe gleich der in der Zerebrospinalflüssigkeit ist, womit der Zusammenhang der Perilymphe und Zerebrospinalflüssigkeit über Aqueductus cochleae bestätigt wird.

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Acta Otolaryng 76: 323–327 1973

HISTOCHEMISCHE SUCCINODEHYDROGENASE-AKTIVITÄT IN DER COCHLEA DES MEERSCHWEINCHENS NACH IMPULSBESCHALLUNG

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(Eingegangen am 20. Februar 1973)

Abstrakt. Nach einer Impulsbeschallung über den Zeitraum von 8 Tagen (täglich 9 h Beschallung mit 16 Impulsen/min, danach 15 h Pause) kam es bei Meerschweinchen zu einem eindeutigen Abfall der Succinodehydrogenase-Aktivität in den Haarzellen sowie den zugehörigen Nervenendigungen. Die Abnahme der SDH-Reaktion war in den äußeren Haarzellen größer als in den inneren. Nach 14tägiger Impulsbeschallung war die Abnahme der Enzymaktivität wesentlich stärker. Die Ergebnisse werden diskutiert.

Nach mehrtägiger Beschallung mit einem Dauerton konnten Vosteen (1958, 1960, 1961) an Meerschweinchencochleae sowie Vinnikow & Titova (1958, 1963) an der Cochlea von Meerschweinchen und Kaninchen eine Abnahme der Succinodehydrogenase-Aktivität in den äußeren Haarzellen nachweisen. Quade & Geyer (1972) fanden nach 63stündiger Einwirkung von 110 dB-„weißem Rauschen" als Dauerlärm im Pausenrhythmus der Industrielärmbelastung einen deutlichen Abfall der Succinodehydrogenase-Aktivität (SDH-Aktivität) in den äußeren und inneren Haarzellen sowie den zugehörigen Nervenendigungen der Meerschweinchencochlea.

In der vorliegenden Arbeit soll untersucht werden, welche Veränderungen der SDH-Aktivität in der Cochlea des Meerschweinchens nach einer Impulsbeschallung auftreten.

Mit Unterstützung durch einen Forschungsauftrag des Ministeriums für Gesundheitswesen der DDR.

MATERIAL UND METHODEN

Es wurden insgesamt 25 Meerschweinchen mit einem Körpergewicht von 250–450 g und mit positivem Preyer-Reflex untersucht. Die Tiere wurden jeweils zu zweit in einem Beschallungskasten untergebracht, wobei jedes Tier einen eigenen Käfig hatte. Von einem Impulsgenerator wurden über eine Funkenstrecke Impulse in den Beschallungskasten gesandt. Die Tiere konnten während der Beschallung Nahrung aufnehmen. Das Beschallungsschema wurde in Tabelle I zusammengestellt.

Nach der Beschallung wurden alle Tiere gleichartig untersucht. Die Präparation und Eröffnung der Bulla tympanica erfolgte in Urethan-Äther-Narkose. Nach Freilegung des runden und ovalen Fensters und Anlegen eines apikalen Bohrloches in die Cochlea wurde ein PVC-Schlauch in das runde Fenster gebracht und bei Bedarf eingekittet. Durch diesen Schlauch erfolgte die Perfusion des Inkubationsmediums. Der SDH-Nachweis wurde mit dem Inkubationsmedium von Ogawa & Barrnett (1965) mit TNBT als Indikator durchgeführt. Unmittelbar nach der Inkubation wurde mit eisgekühlter 4%iger Formaldehyd-0,1 M Phosphatbufferlösung pH 7,4 5 min lang per perfusionem vorfixiert. Zur besseren Strukturerhaltung wurde ein Zusatz

Tabelle I. *Beschallungsschema*

Anzahl der Tiere	Versuchsdauer	Beschallung pro die	Freies Intervall pro die	Impulsfrequenz, Lautstärke
6	—	—	—	—
11	8 d	9 h	15 h	16/min 135 dB
8	14 d	9 h	15 h	16/min 135 dB

von 7,5% Sucrose in die Formaldehydlösung gegeben (Geyer 1969). Im Anschluß an die Vorfixierung erfolgten die Dekapitation und Präparation der Cochlea aus dem Felsenbein. Das Präparat wurde im gleichen Medium 16 h lang bei 4 C nachfixiert. Danach wurde es 14 Tage lang bei 20 C in neutraler Dinatrium EDTA Lösung entkalkt. Das Entkalkungsme dium wurde durch eingeleitete Luftblasen ständig in Bewegung gehalten.

Die Einbettung erfolgte in Polyäthylenglykol 600 nach Halbhuber & Geyer (1966). Am Gefriermikrotom wurden 15 µm dicke Schnitte hergestellt, die in Glyceringelatine ein gedeckt wurden.

ERGEBNISSE

Die Befunde aller 3 Versuchsgruppen sind in der Tabelle II zusammengefaßt.

Nach der Impulsbeschallung kam es im Bereich der gesamten Cochlea zu einer Abnahme der SDH Aktivität. Während die Formazanablagerungen im Cytoplasma der inneren Haarzellen unter Aussparung des Zellkerns bei den unbehandelten Tieren abgesehen von einer gewissen intrazellulären Gliederung nahezu homogen vorlagen (Abb. 1), wurde nach der Beschallung eine inhomogene Ablagerung des Farbstoffs beobachtet. Bei den unbeschallten Kontrollen wurde ein intensives Depot infranukleär befunden, das nach 8 Tagen gering (Abb 2) und nach 14 Tagen (Abb. 3) stark vermindert war. Die Re aktivität im Bereich der Nervenendigungen unter den inneren Haarzellen war erniedr. nach 14tägiger Beschallung konnten d Formazandepots, die den unteren Zellpol b in die Höhe des Zellkerns kelchartig umgr fen nur noch schwach beobachtet werden. Während die Farbstoffablagerungen in d. Nervenfasern nach 8tägiger Behandlung distalwärts kontinuierlich abnahmen und d gleiche Intensität wie die Kontrollen hatten, kam es nach längerer Beschallung zu einem fast vollständigen Aktivitätsverlust.

Die Nervenzellen des Ganglion spirale bl ben in allen Fällen eindeutig negativ.

In den äußeren Haarzellen waren d Farbstoffniederschläge in der Intensität abgestuft. Eine kräftig angefärbte Intermediärzone und eine perinukleär gelegene Zone sowie ei infranukleär befindliches intensives Depot, wie sie bei den Kontrolltieren zu finden waren, konnten nach Impulsbeschallung nicht nachgewiesen werden. Während die Intermediär und die perinukleäre Zone bei den 8tägig beschallten Tieren vermindert erschienen, konnten sie nach 14tägiger Behandlung nur noch verschwommen erkannt werden. Das infranukleäre Formazandepot war nach 8 Beschallungstagen kleiner, nach 14 Tagen kaum noch zu beobachten.

Am unteren Zellpol der äußeren Haarzel-

Tabelle II. *Versuchsergebnisse*

	Kontrolltiere	Beschallte Tiere 8 d	Beschallte Tiere 14 d
Äußere Haarzellen	+ + +	+ +/+ +	
Nervenendigungen unter äußeren Haarzellen	+ + +	+	( )
Innere Haarzellen	+ + + +	+ + +	
Nervenendigungen unter inneren Haarzellen	+ + +	+ +/	+
Tunnelspiralbündel	+	(+)	
N. acusticus	+ + +	+	
Ganglion spirale	–	–	–
Ansatz der Reißnerschen Membran am Lig. spirale	+ + +	+	( )
Lig. spirale	+/+ +	(+)	( )
Prominentia spiralis	+ + +		–
Stria vascularis	–	–	–
Limbus spiralis	+	–	–
Interdentalzellen	+	+	



Abb. 1 SDH-Reaktion am Cortischen Organ eines nicht orbeschallten Meerschweinchens. 750.
Abb. 2. SDH-Reaktion am Cortischen Organ eines Meerschweinchens nach 8tägiger Impulsbeschallung. 750.
Abb. 3. SDH-Reaktion am Cortischen Organ eines Meerschweinchens nach 14tägiger Impulsbeschallung. × 750.

n im Bereich der Nervenendigungen war Enzymaktivität vermindert. Bereits nach Beschallung zeigte sich eine deutliche hme, und nach 14tägiger Impulseinwir reagierten die Nervenendigungen nur h vereinzelt.

Hinsichtlich der Enzymaktivität dominierten in allen drei Versuchsgruppen die inneren über den äußeren Haarzellen. Nach der Impulsbeschallung hatte die SDH Aktivität in den äußeren Haarzellen stärker abgenommen als in den inneren Haarzellen. I in

der Intensität der SDH Reaktion von Haarzellen der basalen und apikalen Windungen konnten nicht festgestellt werden.

Die runden Farbstoffgranula, die sich bei Kontrolltieren entlang der Deiters-Zellen in Richtung Basilarmembran befanden, fehlten bei den beschallten Tieren ganz. Das Tunnelspiralbündel war im Gegensatz zur Kontrolle stets negativ.

Ganz vereinzelt zeigte sich eine Formazanablagerung in den Interdentalzellen sowohl bei der Kontrollgruppe als auch nach 8tägiger Beschallung, während nach 14tägiger Impulseinwirkung keine SDH Aktivität nachweisbar war. SDH-positive Interdentalzellen hatten im gesamten Zelleib reagiert, der Kern blieb ausgespart.

Die geringe SDH Aktivität im Bindegewebe des Limbus spiralis konnte nach Beschallung nicht nachgewiesen werden. Das Ligamentum spirale zeigte normalerweise oberhalb des Ansatzes der Reißnerschen Membran eine deutliche SDH Reaktion, die nach Impulsbeschallung nur noch gering war. Gleichartig verhielt sich das Ligamentum spirale hinter dem Epithel der Prominentia spiralis. Die Stria vascularis blieb in allen Gruppen negativ.

DISKUSSION

In den vergangenen Jahren wurde in einer Vielzahl von Publikationen über Versuche berichtet, die das Studium von Veränderungen des Cortischen Organs nach experimenteller Beschallung zum Inhalt hatten. Mit Hilfe dieser Versuche sollte der Angriffspunkt der Schalleinwirkung an der Schnecke lokalisiert werden.

Vosteen (1958, 1960, 1961) konnte nach mehrtägiger Beschallung mit einem Dauerton eine Abnahme der SDH Aktivität in der Meerschweinchencochlea nachweisen. Die Abnahme der Fermentaktivität erfolgte nach Beschallung mit 2 000 Hz und 70–85 dB zunächst in den Nervenendigungen, danach in den äußeren Haarzellen. Die inneren Haarzellen zeigten gegenüber den äußeren Haarzellen keine Veränderungen.

Über ähnliche Beobachtungen berichteten Quade & Geyer (1972). Sie konnten jedoch einen Aktivitätsabfall in den inneren Haarzellen nachweisen. Im Gegensatz zur Vosteenschen Versuchsanordnung (1958) lagen bei ihrem Beschallungsregime Pausen von 15 *Stunden zwischen den Belastungsphasen*, und die Beschallungsintensität war mit 110 dB wesentlich höher.

In unseren Versuchen konnten wir eine erhebliche Abnahme der SDH Aktivität sowohl in den Nervenendigungen unter den inneren und äußeren Haarzellen, als auch in den Haarzellen selbst beobachten. Die Abnahme der Fermentaktivität war in den äußeren Haarzellen gegenüber den inneren Haarzellen intensiver. Diese Ergebnisse zeigten sich bereits nach 8tägiger Beschallung mit 16 Impulsen/min bei *135 dB und Pausen von 15* Stunden zwischen den Belastungsphasen. Werden die Meerschweinchen 2 bis 4 Tage bei 2 000 Hz und 85 dB mit Kurztönen von jeweils 250 msec Dauer beschallt, so kommt es nach Vosteen (1958) zu einer geringen Abnahme des Fermentgehaltes der äußeren Haarzellen, während die inneren Haarzellen und Nervenendigungen eine gute Reaktivität behalten.

Vergleicht man die Schallintensität und Beschallungsdauer mit dem Aktivitätsverlust der SDH, so korrelieren unsere Ergebnisse mit denen von Vosteen (1958, 1960, 1961) sowie von Vinnikow & Titova (1958, 1963) gut.

Nach Vosteen (1958) soll dem Untergang der äußeren Haarzellen nach Beschallung ein Schwund der SDH vorausgehen. Der Rückgang der SDH Aktivität soll einen Erschöpfungszustand nach funktioneller Überanspruchung zum Ausdruck bringen. Die Abnahme der SDH Aktivität in den Haarzellen und ihren Nervenendigungen ist wahrscheinlich mit einer Hypoxydose kombiniert, der für das Entstehen der Schädigung eine wichtige Rolle zugesprochen wird (Mishrahy et al. 1957, Vosteen, 1961, Beck & Beickert, 1959).

Zu Zerreißungen und Zerstörungen des Cortischen Organs kommt es nach Rüedi & urrer (1947) wenn der Schalldruck bei 140 dB liegt. Da unsere Versuche mit einer Schall- ntät von 135 dB ausgeführt wurden könnte eine Zerstörung des Cortischen Organs auftreten. Möglicherweise wird der schalltrau matische Effekt jedoch durch die Beschallungspausen von durchschnittlich 3 7 sec zwi- en zwei Impulsen verringert. Nach Vosteen (1958) ist auch nicht die Summe der Schall- ntitäten in erster Linie für die Erschöpfung der Sinneszellen verantwortlich, sondern vielmehr die Kontinuität der Beschallung. Dafür spricht das Ergebnis unserer Untersu ebenfalls.

Mit den histochemischen Befunden ste en elektronenmikroskopische Beobachtungen Veränderungen an den Mitochondrien in guter Übereinstimmung (Spoendlin, 1958, 1970, 1971). Sie treten nach kurzzeitiger Be stung mit 100–130 dB- weißem Rauschen" den Nervenendigungen und in Haarzellen Anschließend lassen sich osmiophile n se in den Lysosomen als Ausdruck generativer Abbauprozesse nachweisen.

SUMMARY

In guinea pigs succinic dehydrogenase activity of hair s and of their nerve endings had decreased significantly after exposure to impulse sound for 8 days 16 impulses per minute during 9 hours each day 15 hours rest between every period of sound ex osure) The decrease of the enzyme activity was ore pronounced in the outer hair cells of the organ Corti than in the inner hair cells. An identical reatment for 14 days considerably enhanced the ef ect The authors discussed the meaning of their findngs

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Acta Otolaryng 76, 328–330, 1973

DISCREPANCY BETWEEN THE LOUDNESS FUNCTION AS MEASURED BY THE ABLB AND THE METZ METHOD

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(Received February 12, 1973)

Abstract. In a patient with acoustic neuroma we found a distinct and well documented discrepancy between the loudness function as measured by the ABLB test and that measured by the Metz test. It is the first such case that has been published and with our present knowledge it is not possible to explain this finding. The inevitable conclusion is that the phenomena that determine subjective loudness sensation and the middle ear muscle reflexes are not totally identical.

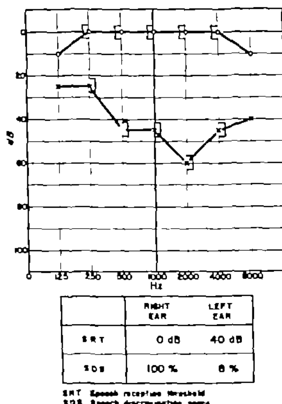
It is generally recognized that the acoustic middle ear muscle reflexes are determined by the subjective loudness sensation. This forms the basis for the use of these reflexes as a test for recruitment of loudness, the socalled Metz test. In clinical materials there is very good agreement between the results obtained by the Metz test and the classical ABLB test. This has been described previously by Thomsen (1955) as well as by Ewertsen et al. (1958). In psychophysical experiments Müller (1970) confirmed these findings. The purpose of this paper is to present a case in which there is a distinct discrepancy between the outcome of these two methods. To our knowledge this is the first such case that has been reported.

Case Report

A 40-year-old female who was admitted to our department for a left acoustic neuroma. During the past 2 years she had noticed a progressive loss of hearing on the left ear. Caloric vestibular tests by the Hallpike method showed no reaction whatsoever on the left labyrinth. There was preponderance to the right, indicating a recently developed abolition of the reaction on the left side. No spontaneous or positional nystagmus was observed, and the optokinetic test showed normal findings. The facial and the trigeminal nerves were not affected. Tomography showed enlargement of the internal porus of the left side and Iophendylate cisternography demonstrated a smooth round tumour with a diameter 12–13 mm at the internal porus on the left side. The tumour was removed by a posterior fossa approach. Its magnitude was as expected from the roentgenological studies. Anteriorly it reached the trigeminal nerve. The tumour gave rise to a small impression in the pons but there were no adhesions to the brain-stem. The facial nerve had to be cut, but otherwise the operation was completely successful.

Audiological findings

Pure tone audiograms showed a sensorineural hearing loss on the left ear (Fig. 1) and normal hearing on the right ear. Békésy tracings on the left ear were not typical and according to Jerger (1960) they must be classified as a borderline case between type II and type IV. The ABLB test (Fig. 2) shows almost complete recruitment of the delayed type for 1 000 and 2 000 Hz. These results were reproduced several times with great precision, and variations



	RIGHT EAR	LEFT EAR
SRT	0 dB	40 dB
SDS	100 %	8 %

Fig. 1. Pure tone audiogram and speech tests.

were within ±5dB. On the left ear we recorded a normal middle ear muscle reflex with a 1 000 Hz tone applied to the right ear. The threshold was 85 dB, which is within the normal range. When tones were applied to the left ear there was no reflex on the right ear even with intensities of 120 dB at 500, 1 000 and 2 000 Hz. With the use of a Bárány noise box held close to the ear, but without touching the skin, however, we observed a normal reflex. Because of the unsatisfactory control of the sound intensity from the noise it was not possible to decide if there was a pathological reflex decay as described by Anderson et al. (1969).

DISCUSSION

From a diagnostic point of view this case is without interest. The preoperative findings are in good agreement with the presence of an acoustic neuroma. Even the presence of recruitment by the ABLB test is not unusual. Thus Johnson (1972) in a series of 521 patients with acoustic neuroma found a positive ABLB test in 23% of those that were tested by this method. The only point of interest is the discrepancy between the outcome of the ABLB and the Metz test.

It is not advisable to draw conclusions from such findings in a single patient without a discussion of the validity of the results. The ABLB test was performed by means of the Madsen ZO-70 audiometer, which has an automatic timing device for this procedure. The test was carried out at various frequencies and on several occasions by different investigators. The patient was able to indicate the level of loudness equality within variations on the pathological ear of only 5 dB and all our results are practically identical. The patient has a delayed type of recruitment, but it is irrelevant in this connection. The important thing is that the subjective loudness sensation at these high sound levels where we should expect an acoustic middle ear muscle reflex is almost the same on the two ears. The absence of a middle ear muscle

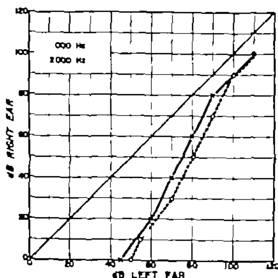


Fig. 2. ABLB-test, results.

reflex, when the pathological ear was stimulated with pure tones attains a high degree of significance by the finding that such a reflex was present when even stronger acoustic stimuli were applied. The reflexes recorded under these conditions were of the ordinary positive going type and were not accompanied by startle reactions. In order to ascertain identity between the stimuli at the ABLB and the Metz tests we used the Madsen ZO-70 audiometer also during reflex measurements. The maximum tonal output from this audiometer is 120 dB and it did not suffice to elicit a reflex. The Bárány noise box reaches intensities of 130–140 dB in the 1 000 Hz frequency area. The contralateral ear was normal in all respects and here the reflex threshold was 85 dB at 1 000 Hz. Thus it was necessary to apply sounds that were at least 40 dB stronger in the pathological ear in order to attain a reflex in spite that the ABLB test showed almost complete recruitment of loudness. The difference is so large that it falls far outside the normal interaural variations for the acoustic reflex thresholds, and it seems justified to state that in this particular case the mechanisms that determined the subjective loudness ion and the acoustic middle ear muscle reflexes are at a variance

Our knowledge about the anatomical side of the socalled final or central loudness evaluation is uncertain, but there is evidence that it is located in the brain stem, as suggested by Djupesland & Zwislocki (1971) The acoustic middle ear muscle reflexes have a very short period of latency thus Perlman & Case (1939) found an average value of 10.5 msec and there can be no doubt that the basic central evaluation underlying these reflexes also is performed in the brain-stem. The neuroma in our patient was large enough to cause disturbances in the function of this structure and thus possibly accounted for the discrepancy between these two mechanisms, which otherwise are so well coordinated. But just the fact that these two mechanisms under certain circumstances can be separated appears to prove that they at least are not totally identical.

ZUSAMMENFASSUNG

Bei einem Patienten mit Acusticus-Neurinom haben wir eindeutig eine Nichtübereinstimmung der Stärkefunktion gefunden die einerseits mit der ABLB-Probe und andererseits mit der Metz Probe gemessen wurde Es ist das erste Mal, dass ein Fall dieser Art publiziert wird. Beim jetzigen Stand unserer Kenntnisse ist eine Klärung jedoch noch nicht möglich. Es muss gefolgert werden, dass die Erscheinung, die subjektives Stärkegefühl vermittelt und die Mittelohrmuskelreflexe bedingt nicht total identisch ist.

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Acta Otolaryng 76 331–338, 1973

MORPHOLOGY OF THE VESTIBULAR NERVE

III. *Analysis of the Calibers of the Myelinated Vestibular Nerve Fibers in Man at Various Ages*

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Abstract. An analysis of the calibers of the preganglionic portion of the myelinated vestibular nerve fibers in man at various ages has been made. In all 10 210 fibers were analysed. The material consisted of nerve specimens from one newborn, 4 adults with "normal" or near normal" numbers of vestibular fibers and 4 old persons with distinct reductions in number of nerve fibers. In the newborn the fibers were generally thinner than in the adult and old persons. The ampullary nerves and especially the two superior branches, contained proportionally more thick fibers than the macular nerves. In the old age group the proportion of thick fibers was less than in the adult group this was particularly evident in the ampullary nerve branches. It could not be determined whether this was caused by disappearance of the thick fibers or was the result of a general involution of all nerve fibers in old age

The myelinated vestibular nerve fibers in man have been found to suffer a significant reduction in number with increasing age (Bergström, 1972). Preliminary observations in that study indicated that the thickest fibers in the ampullary nerve branches were particularly affected by this reduction.

It has also been shown that the sensory cells on the cristae are relatively more richly innervated than the macular hair cells (Bergström & Engström 1973).

The caliber variations in the vestibular nerve have previously been analyzed by Engström & Rexed (1940) who reported that 88.5% of the fibers had an outer diameter of 2–8 microns. 7.2% were thicker than 8 microns and 4.2% thinner than 2 microns. Rasmussen (1940) stated that the vestibular fibers varied from 2 to 15 microns with the majority being at least 10 microns thick.

In the present study the outer diameters of the myelinated fibers in the different vestibular nerve branches have been measured on nerve specimens obtained from individuals of various ages. The aim of the investigation was to clarify the caliber spectrum of the myelinated fibers in the ampullary and macular branches and to see whether any variations occur with old age and with the concomitant decrease in number of nerve fibers.

MATERIAL AND METHODS

The investigation was performed on cross-sections from the preganglionic parts of the vestibular nerve branches. The material was obtained from 9 cases included in the previous numerical study Methods of fixation, embedding and staining are presented in that paper (Bergström, 1973).

The material was divided into three groups, the first of which was a newborn boy with 18 773 vestibular nerve fibers. The second group consisted of 4 young to middle aged persons with "normal" or near "normal" numbers of vestibular nerve fibers (16 040–18 359) and the third group consisted of 4 old persons, all with markedly reduced numbers

This work has been sponsored in part by NASA Grants No. NGR 52-028-003 and NGR 52-028-004 and the University of Uppsala.

Table I *Age and total number of vestibular nerve fibers in the caliber analysed cases*

Newborn	Adults	Old
1 day 18 773	22 yrs, 18 359	78 yrs, 9 274
	35 yrs, 16 040	80 yrs, 10 205
	49 yrs, 16 582	80 yrs, 10 074
	53 yrs, 16 817	85 yrs, 1[illegible] 000

Table II *Number of caliber analysed fibers in the different vestibular nerve branches and age groups*

	Newborn	Adults	Old
Nn amp ant et lat	892	747	1 456
N amp post	—	769	1 046
N utric	946	762	1 140
N sacc	344	1 059	1 049
	2 182	3 337	4 691

of nerve fibers (9 274–12 000) The individual nerve populations are shown in Table I The mounted nerve sections were photographed with a Leitz Orthomat camera through an Orthoplan microscope at 128 × magnification on Adox KB 14 and Ilford Pan F film. The copies were enlarged to 1 000 × magnification and thus 1 mm on the copy corre[illegible]nded to 1 micron in the specimen. At this magnification the outer diameters of the nerve fibers could be measured with accuracy At least two diameters were measured, account was made for disfiguration of fibers closely packed and, on irregularly contoured fibers, the most natural looking diameter was used. The most distorted fibers were excluded. Fibers ranging in diameter from 0.6 to 1.5 microns are said to have a diameter of 1 micron, 1.6 to 2.5 are reported as 2 microns thick etc. A total number of 10 210 fibers were analysed. Distribution by age groups and nerve branches is seen in Table II In the newborn case the sections from the posterior ampullary nerve were not sufficiently flattened out under the cover glass to allow proper focusing for photography and the nerve branch was excluded. Each photograph contained 100–500 fibers (300 on average) and covered different parts of the cross-section of the nerve branches so that both peripheral and central fibers were included.

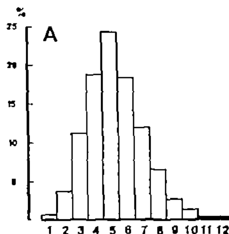
OBSERVATIONS

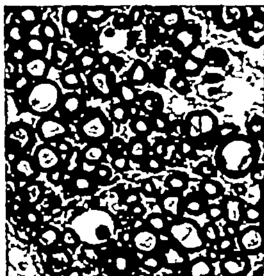
Superior ampullary nerves

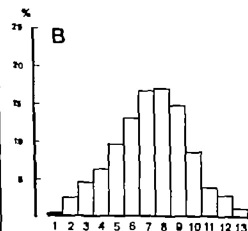
Since the nerve sections are from a level immediately distal to the vestibular ganglion

[illegible]able III *Distribution of fiber diameters in the different vestibular nerve branches in various age groups. Expressed in*

Diam. in µm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Naa																
Newborn	0.6	3.6	11.[illegible]	18.8	24.4	18.4	11.9	6.5	[illegible]7	1.3	0.3	0.3				100.0
Adults	0.3	[illegible]3	4.4	6.1	9.5	13.0	16.7	17.0	14.8	8.5	3.7	[illegible]7	1.0			100.0
Old group	0.9	1.6	6.[illegible]	10.7	18.7	19.9	14.8	11.0	8.5	4.5	[illegible]0	0.7	0.4	0.1		100.0
Nap																
Adults	0.4	0.2	4.7	8.7	16.4	18.0	15.6	14.0	9.8	6.4	3.0	1.3	1.1	0.3	0.1	100.0
Old group	[illegible]2	4.3	11.4	18.3	20.6	15.9	12.0	8.6	4.9	1.2	0.1	0.4	0.1			100.0
Nutric																
Newborn	1.3	8.6	18.9	[illegible]	20.5	11.8	8.7	5.7	1.9	1.3	0.1	0.2				100.0
Adults	1.0	3.3	8.1	10.1	15.9	19.3	17.9	13.4	7.1	3.1	0.4	0.1	0.1	0.[illegible]		100.0
Old group	1.3	[illegible]3	5.8	13	23.1	18.8	13.5	8.9	6.0	3.3	1.9	1.2	0.3	0.3	0.1	100.0
Nsacc																
Newborn	[illegible]9	[illegible]3	10.[illegible]	20.1	25.8	17.1	9.6	6.4	3.2	0.9	0.6	0.9				100.0
Adults	1.3	1.9	5.1	13.3	19.1	17.1	14.2	1[illegible]6	8.6	4.[illegible]	1.8	0.6	0.[illegible]			100.0
Old group	1.[illegible]	[illegible]4	8.0	15.8	23.4	[illegible]1.5	14.8	6.0	3.9	2.3	0.3	0.4				100.0









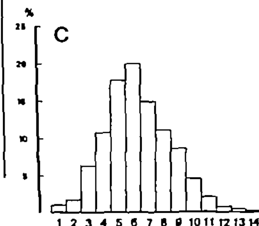
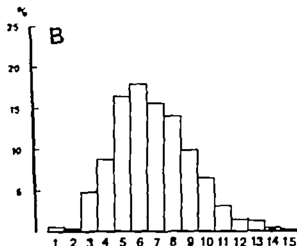
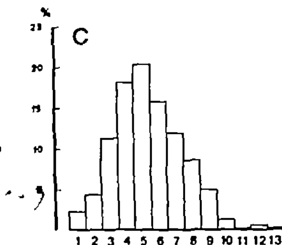




Fig. 1 Diagrams of caliber spectra with diameters in microns. (A) newborn, (B) adult group (C) old age group. Nerve section from corresponding age group to the right of diagram. Anterior and lower nerve. ×1000







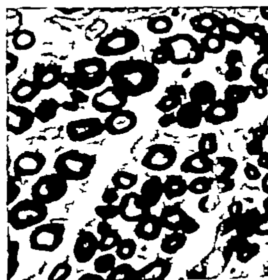


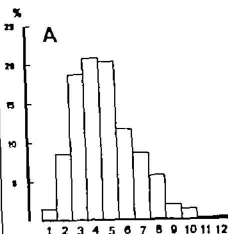
Fig Diagrams of caliber spectra with diameters in microns (B) adult group, (C) old age group. Nerve section from corresponding age group to the right of diagram. Posterior ampullary nerve 1000.

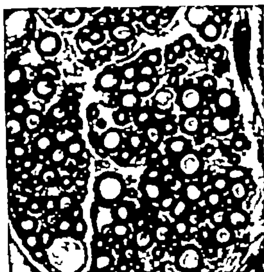
where the lateral and anterior ampullary nerves are inseparable they are presented as a unit.

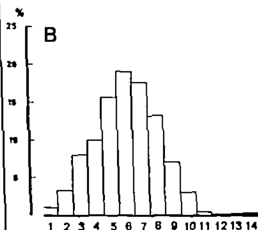
Newborn The fiber diameters range from 1 to 12 microns with the majority 61 6 being 4 to 6 microns. Of the fibers, 23 are thicker than 6 microns. In this case there are slightly more thin fibers (1 to 3 microns) in the inferior part of these nerve branches than in the superior

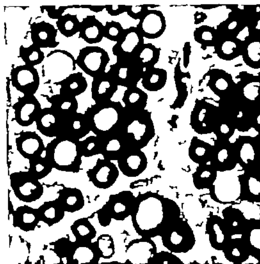
Adult group. In this group with "normal" fiber counts the majority of the fibers are thick. Diameters of 61 5% are from 6 to 9 microns and 15 9% of the fibers are thicker than 9 microns.

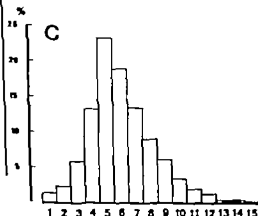
Old age group. Fibers from 4 persons in this age group were analysed. In 1 case an 80-year old, the superior ampullary nerves could not

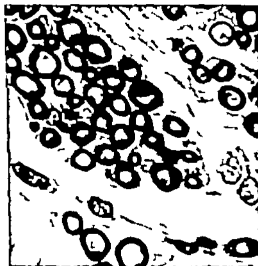




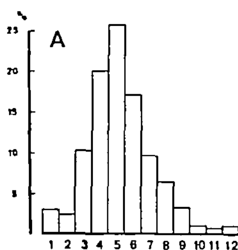


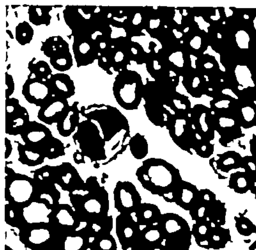


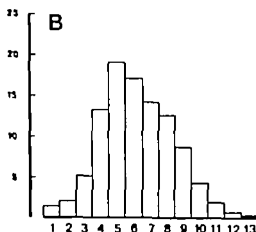




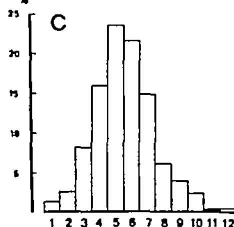
. 3. Diagrams of caliber spectra with diameters microns. (A) newborn, (B) adult group, (C) old age group. Nerve section from corresponding age group to the right of diagram. Utricular nerve. 1 000.











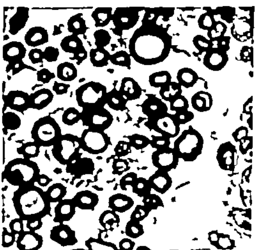


Fig. 4. Diagrams of caliber spectra with diameters in microns. (A) newborn (B) adult group (C) old age group. Nerve section from corresponding age group to the right of diagram. Saccular nerve. 1000

be reliably demarcated from the utricular nerve, therefore the fibers of the superior division were presented as a unit in the numerical study The area chosen for the caliber analysis in this case is from the superior part of the cross-section and well inside the ampullary nerve region.

Compared with the adult group there is a deviation towards the thinner calibers. Of the fibers, 54.2% have diameters from 6 to 9 microns and only 7 7% are thicker than 9 microns.

Posterior ampullary nerve

Adult group. In this ampullary nerve branch the thick fibers are also predominant. The diameters of 54.2% are from 6 to 9 microns and 12.2% are thicker than 9 microns. The individual variations are rather pronounced especially concerning the thickest fibers.

Old age group. With old age and the subsequent reduction in the number of nerve fibers a distinct loss of thick fibers is evident. Of the fibers in this age group 41 4% are found within the 6 to 9 microns range and only 1.8% are thicker than 9 microns. The individual variations are small in this group.

Utricular nerve

Newborn. In this macular nerve branch the fibers are generally thinner than in the superior ampullary nerves. The diameters of 53.4% are between 4 and 6 microns and 19.9% are thicker than 6 microns.

Adult group The same situation is found in this age group with the utricular nerve fibers being thinner than the ampullary fibers although a few fibers as thick as 14 microns are observed. Of the fibers, 57 7% are between 6 and 9 microns in diameter but only 3 9 % are thicker than 9 microns.

Old age group. A loss of the thicker fibers occurs also in this nerve branch. 47.2% of the fibers have diameters from 6 to 9 microns but 7 1% are thicker than 9 microns, although there are individual variations.

Saccular nerve

Newborn. In this newborn boy the saccular nerve fibers show approximately the same caliber variations as the fibers of the superior ampullary nerves. 63% of the fibers are 4 to 6 microns thick and 21 6% have greater diameters than 6 microns.

Adult group In this age group with "normal" numbers of nerve fibers the caliber distribution is similar to that of the utricular nerve 52.2% of the fibers have diameters from 6 to 9 microns and 6.8% are thicker than 9 microns.

Old age group. A clear reduction of the thicker fibers is observed with 46 2% of the fibers in this group measuring 6 to 9 microns and only 3 0% having diameters greater than 9 microns. The individual variations are pronounced in this group

DISCUSSION

The ampullary nerves and especially the two superior branches, contain a greater proportion of thick fibers than the macular nerves. The hair cells on the cristae are relatively more richly innervated than those on the maculae and they are also innervated by proportionally more thick fibers than the macular cells. The conduction velocity of the nerve fiber is nearly proportional to the diameter (Rushton, 1951). These observations imply that there are greater demands for fast reactions to angular than to linear accelerations.

Individual variations regarding caliber conditions are found in the "normal" group the 22-year-old woman with 18 400 vestibular nerve fibers has more of the thickest fibers (9 microns and more) than the average in all of the nerve branches analysed, while the 35-year-old man with 16 000 fibers has less of these thick fibers than the average

In old age a reduction in number of vestibular nerve fibers occurs. In the present study a deviation of the caliber spectrum towards the thinner side has been shown in the old age group These changes are most pro-

nounced in the ampullary nerve branches. Whether the thick fibers disappear first or the change in caliber pattern is the result of a general involution of all nerve fibers in old age cannot be determined. The vestibular nerve fibers from a newborn boy have also been analysed and they were generally thinner than in the adult cases and the nerve fibers from a 6-week-old boy included in the previous numerical study were also generally thinner than those from the adults. It has been found earlier (Westphal, 1897) that there is a gradual increase in the size of the myelin sheath with increasing age. Embryological studies (Langworthy 1933) have shown that myelinization of the vestibular nerve fibers begins at the 20th fetal week and the process is not completed until the time of puberty. The differences in caliber spectra between the newborn and the adults in the present study can well be explained against this background.

ZUSAMMENFASSUNG

Eine Analyse der Kaliberverhältnisse bei den myelinisierten, praganglionaren Vestibularnervenfasern bei Menschen verschiedenen Alters wurde durchgeführt. Insgesamt wurden 10 210 Fasern analysiert. Das Material bestand aus Nervenpräparaten eines Neugeborenen, von 4 Erwachsenen mit "normaler" oder "fast normaler" Anzahl Vestibularnervenfasern und 4 alten Personen mit deutlich reduzierter Anzahl Nervenfasern. Bei dem Neugeborenen waren die Fasern durchgehend dunner als bei den Erwachsenen und alten Personen. Die Ampullarnerven und besonders die beiden oberen Äste enthielten verhältnismässig mehr dicke Fasern als die Maculanerven. In der Gruppe der alten Personen war der Anteil dicker Fasern geringer als in der Gruppe mit normalen Nervenfasern. Dies war besonders deutlich bei den Ampullarnervenästen. Es konnte nicht festgestellt werden, ob dies durch ein Verschwinden der dicken Fasern verursacht wurde oder das Ergebnis einer allgemeinen Involution aller Nervenfasern im Alter war.

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Acta Otolaryng 76: 339–348, 1973

OPTOKINETIC MOTION SICKNESS AND PSEUDO-CORIOLIS EFFECTS INDUCED BY MOVING VISUAL STIMULI

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(Received February 27, 1973)

Abstract. Pseudo-Coriolis effects (PCE) and optokinetic motion sickness are elicited by bending the head out of the axis of rotation of a circular visual surround when that moving surround induces the illusion of self-rotation. With respect to tilt sensation and vegetative symptoms, optokinetic PCE correspond to vestibular Coriolis effects (CE) that arise from similar head movements when the body is actually rotating. Quantitatively, PCE are of smaller magnitude than CE and saturate at lower velocities of stimulation (90–120°/sec). PCE, depending on the illusory sensation of self-rotation (circularvection, CV), share its prolonged time course after stimulus onset and termination, its relation to stimulus velocities and its dependency on stimulus area with predominance of the retinal periphery. Optokinetic influences on vestibular CE depend on the direction and speed of the moving visual stimulus and result in either inhibition or facilitation of apparent tilt and nausea.

Large moving visual scenes can induce the perception of self-motion opposite in direction to the moving visual stimulus and it has been reported that such stimulation can cause motion sickness in the absence of actual body movement. The sickness has been reported in immobile people viewing a cylinder rotating around them (Crampton & Young, 1953), in wide-screen movie theatres (Money, 1970) and in a helicopter simulator that included a large moving visual display (Miller & Goodson, 1960). The mechanisms through which this kind of motion sickness arises, however, have not yet been elucidated.

It is the aim of this paper to describe in detail what we call *pseudo-Coriolis effects (PCE)* and to demonstrate that *optokinetic motion sickness* can be elicited by repeated provocation of PCE. It will be stressed that PCE cannot subjectively be distinguished from true Coriolis effects (CE) although the stimulus conditions are totally different. CE arise from bending the head during real body rotation. PCE, by contrast, are elicited by bending the head during an apparent self-rotation, called circularvection (CV), that is provoked by a large visual surround rotating about a vertical axis around a stationary observer. Thus, PCE do not require the labyrinthine cross-coupling effects that arise from similar head movements during real body rotation. But they require the sensation of motion which is not necessary for true CE. It will be shown that two components are necessary to elicit PCE. One is a central equivalent of vestibular excitation mediated by visual motion information, the other is the vestibular input generated by the head movement itself.

The description of PCE will entail data on the time course in relation to stimulus onset and stimulus end and the importance of stimulus speed and stimulus area and location within the visual field. It will be supplemented by some results on the influence of optokinetic stimulation upon the strength of Coriolis effects that not only suggest practical applications but also led us to a preliminary hypothesis on the possible neurophysiological mechanisms underlying this interaction.

This work was supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 70 („Hirnforschung und Sinnesphysiologie").

METHODS

Subjects

A total of 89 students (39 females, 50 males) took part in 8 series of experiments. Subjects were previously unfamiliar with the phenomena investigated and were paid for their participation.

Apparatus

Subjects sat on a rotating chair located in the centre of a closed cylindrical drum 1.5 m in diameter whose inner walls were painted with vertical alternating black and white stripes each subtending 7° of visual angle (Tönnies/Freiburg). The chair and the drum could be rotated separately or simultaneously at the same or different speeds up to 180°/sec in either the same or opposite directions. During combined rotation of both drum and chair at identical speeds, drum and chair were connected by means of a clutch. Acceleration, speed (servo-controlled), and angular position of both drum and chair were continuously recorded.

Optokinetic stimulation by the moving drum could be restricted to any desired spatial extent and could be presented at any location within the visual field. This was achieved by using black masks which were mounted immediately adjacent to the inner wall of the drum. The masks were fixed on poles connected to the back of the chair. To stabilize the direction of the visual axis, subjects were asked to fixate a 1° luminous spot mounted on the chair and presented in a position 60 cm straight ahead of the subject.

The subject's head was restrained by a head-holder that allowed the subject to bend his head 45° towards the shoulders when a hinge-like joint at its base was loosened.

Recordings

Horizontal and vertical eye movements were separately recorded by means of electro-oculography (Jung, 1953) and displayed on a strip-chart recorder. In some of the experiments, electro-cardiograms (ECG), respiration (using a thermo-element) and galvanic skin responses were recorded as well. Blood pressure was measured repeatedly.

Quantifications

Subjects, unaware of the real stimulus conditions, were asked to indicate whether they perceived self rotation or surround motion or both. Latencies from the sudden onset (lights on) of constant velocity optokinetic stimulation to the onset and completion of perceived self-motion, as well as the duration of perceived self-rotation after stimulus end (lights off), were measured by the subjects using stop-watches. Velocities of real or illusory self-motion and intensities of apparent body tilt and nausea in CE and PCE resulting from head movements were scaled separately by magnitude estimations (Stevens, 1957). Scalings were performed in reference to a standard stimulus (modulus) presented once at the beginning of the experiment. The modulus was assigned an arbitrary value. In addition, subjects were asked about the direction of apparent tilt. Comparisons of CE and PCE were supplemented by the recordings of blood pressure and galvanic skin resistance. In the experiments on acute optokinetic and vestibular motion sickness, the number of head movements necessary to provoke acute motion sickness, nausea and vomiting or retching, was determined. Up to 40 head movements were requested at 10-second intervals. The time course of nausea symptoms was followed over a period of 24 hours.

Experimental procedure

Before beginning the experiment, subjects were told about all the possible stimulus situations, but were not informed of the actual stimulus. Subjects first practised bending the head sideways over the whole range of 45° allowed by the head-holder. The head movement initially to the right and then to the left shoulder was to be completed within 4

seconds following the experimenter's command.

In the experiments, head movements were performed only during constant velocity rotation of either chair or drum or combination of both that were presented randomly. As a rule, one sequence of two head movements first to the right and then to the left was requested 15 sec after constant velocity was reached, following a period of acceleration at less than 0.9 /sec². Between trials, the drum was opened for a 2-minute period of relaxation.

RESULT

Qualitative similarities of CE and PCE

Pseudo-Coriolis effects (PCE) may be elicited by bending the head during an apparent self rotation induced by rotatory motion of the visual environment. PCE also occur in subjects without prior experience of Coriolis effects in the experimental arrangement. Neither apparent self-rotation nor PCE could be distinguished from real body rotation and CE respectively. The perceptual effects of PCE and the associated vegetative symptoms qualitatively corresponded to those of the well-known Coriolis effects (CE). Apparent tilt, combined with a yawing sensation, nausea, increase in heart rate and irregularity of respiration in CE and PCF showed no qualitative differences (Brandt et al. 1971). Even the perceived directions of apparent tilt in CE and PCE seemed to correspond provided that head movements were performed in the same direction and during identical directions of perceived self-rotation.

Optokinetic motion sickness

The experiments summarized in Fig. 1 demonstrate that *acute motion sickness can be elicited* through PCE, i.e. *in the absence of real body motion*. In checking the overt symptoms of motion sickness (pallor, sweating, hypersalivation, disorientation, nausea, drowsiness and vomiting), no qualitative difference could be found between the two extreme con-

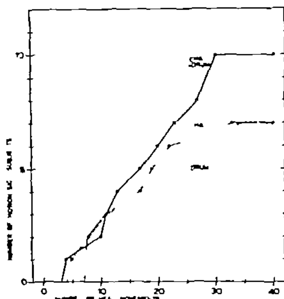


Fig. 1 Cumulative frequency of acute motion sickness (*ordinate*) in relation to number of head movements (*abscissa*) performed during exclusively optokinetic stimulation (drum rotation, only DRUM), optokinetic and vestibular stimulation (chair rotation, drum stationary CHAIR) and vestibular stimulation without visual motion clues (combined chair and drum rotation, CHAIR + DRUM). Rotations at 90°/sec, constant velocity

ditions of provocation: body rotation without surround motion (drum and chair rotating together) or drum rotation with the body stationary. Quantitatively the symptoms of motion sickness elicited by visual stimuli are either less intense or a more prolonged stimulation is required to elicit acute motion sickness. Aftereffects consisting of prolonged nausea, drowsiness and dizziness, which were elicited by rapid head movements, also occurred in optokinetic motion sickness and often lasted the rest of the day.

Quantitative differences of CE and PCE

Apparent tilt and nausea are weaker for PCE Fig. 2 A 2, -4) than for CE provided that CE are elicited during chair rotations with the eyes closed (Fig. 2 A 1) or the visual environment rotating with the chair (Fig. 2 A-6). Under the latter condition there are no clues about motion from the visual environment.

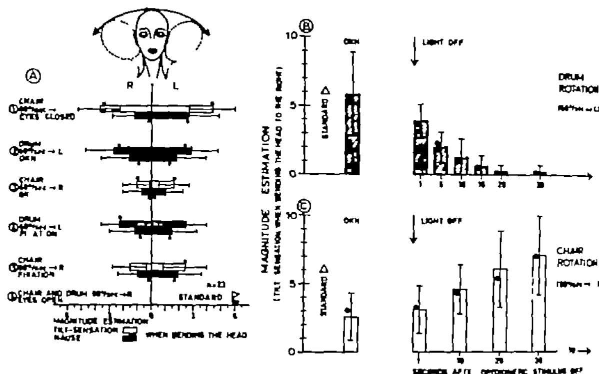


Fig 2 (*A*) Magnitude of tilt sensation and nausea (*abscissa*) in CE and PCE. Stimuli and conditions of observation are listed on the left. (B) Decrease in magnitude of PCE 1–30 seconds after the end of a 30 second optokinetic stimulation (lights off). (C) Decrease in optokinetic inhibition of CE 1–30 seconds after lights off during chair rotation. (Means and standard deviations = columns; medians = black dots)

As soon as the rotating subject views a visual surround that is stationary in space, CE (Fig. 2 A 3 5) are weaker than PCE. In these cases, the presence of visual motion stimulation (motion of the visual surround relative to the subject) obviously inhibits CE. The differences above described for tilt are significant on the 0.02 level (Wilcoxon test) The magnitudes of CE and PCE are not significantly influenced by whether or not eye movements (OKN) occur during visual motion stimulation. Nevertheless, it has been found in other experiments that PCE repeatedly were stronger with optokinetic nystagmus than with fixation

Time course of PCE and optokinetic inhibition of CE

Fig. 2 B shows an almost exponential decrease of PCE after the end of the visual stimulus. PCE can be elicited up to 30 sec after stimulus termination thus resembling CV in its time course (Brandt & Dichgans, 1972, Brandt et al. 1973) The time course of susceptibility to PCE after stimulus end is paralleled by that of optokinetic inhibition after the end of the visual stimulus reflecting the decreasing optokinetic inhibition The optokinetic inhibition outlasts the termination of the visual stimulus up to 30 sec in a few subjects (Fig. 2 C)

Fig 3 shows the delayed increase in the magnitude of tilt perceived during provocation of PCE after onset of optokinetic stimulation. Apparent tilt during PCE increases up to at least 30 sec after stimulus onset, showing a more prolonged time course than does the visually mediated sensation of self rotation (CV), which reaches its steady state after an average of 6 sec among these subjects.

Relation to stimulus velocity

Magnitude of CE and PCE increases with the angular velocity of chair and drum rotation

respectively (Fig. 4) CE elicited by head movements during eye closure increase almost linearly up to 120 /sec of chair rotation, the highest velocity tested. PCE seem to reach saturation between 90 and 120 /sec of drum rotation. The leveling-off of PCE at higher velocities corresponds well to earlier results of Brandt et al. (1973) demonstrating that the visually induced sensation of self motion saturates at approximately the same velocity

Directional specificity of optokinetic inhibition of CE

Only optokinetic stimulation in the opposite direction to chair rotation mediates a sensation of self-rotation that perceptually agrees with the body movement and is capable of inhibiting CE (Fig. 5) Optokinetic stimulation which induces a sensation of self rotation opposite in direction to the real rotation enhances CE. Within a velocity range of ±60 / sec, in both directions, the interaction between optokinetic stimuli and magnitude of CE, elicited during a 60 /sec chair rotation, is almost linear The fact that inhibition shows no further increase with optokinetic stimuli moving at more than 90 /sec opposite in direction to the actual body movement might be explained by the relation between speed of optokinetic stimuli and magnitude of self motion sensation (cf Brandt et al. 1973). The enhancement of CE by optokinetic stimulation in the same direction is so strong that, with a 60 /sec chair rotation and a 120 /sec drum rotation, several subjects became motion sick and vomited after one head movement.

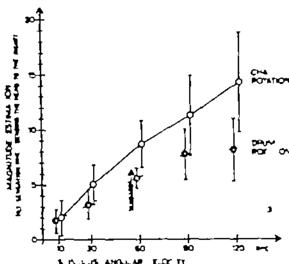


Fig. 4 Magnitude of perceived tilt in CE ( ) and PCE ( ) in relation to angular velocity of chair rotation in the dark and drum rotation with OKN respectively (Means and S.D)

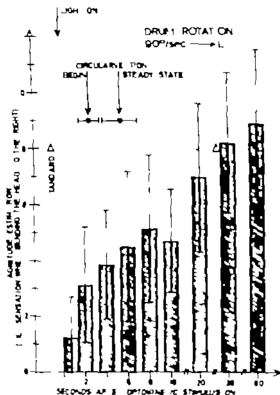


Fig. 3 Magnitude of perceived tilt in PCE (*ordinate*) in relation *to time after onset of the optokinetic* stimulus (abscissa). For comparison, note the latencies of begin and steady state of circularvection. (Means and standard deviations).

Magnitude of PCE depends on stimulus area and location within the visual field

Fig. 6 depicts magnitude estimations of the subjective velocity of the visually induced sensation of self-rotation and scalings of tilt in PCE. The strong relationship between circularvection and PCE is suggested not only by

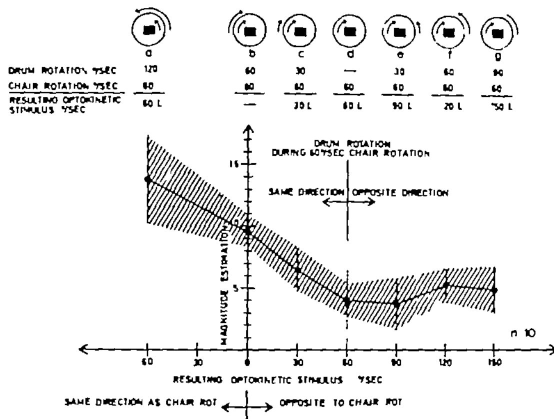


Fig. 5. Inhibition and facilitation of CE (*ordinate*) in relation to direction and speed of the optokinetic stimulus (*abscissa*) combined with a constant velocity chair rotation of 60°/sec. Stimuli are listed in the upper part. (Means and S.D. of perceived tilt).

ilarities in time course and relation to stimulus velocity but also by the marked co-variation of both in relation to stimulus area and its location within the visual field ($r = 0.785$ $p2\alpha \leq 0.002$ [df = 25]). PCE and circularvection similarly increase with an increase in the stimulus area and are stronger for stimulation of the peripheral retina. Masking the centre of the visual field up to 120° in diameter (Fig. 6 *d*, *e*) scarcely diminishes either sensation, whereas stimulation of the centre (Fig. 6 *f*, *g*) barely elicits them. The relative independence from stimulation of the centre of the visual field is further demonstrated in Fig. 6 *k* where central (30° in diameter) and peripheral stimuli move in opposite directions. In this instance circularvection and PCE are determined by the peripheral stimulus, whereas optokinetic nystagmus relies on the central stimulus.

DISCUSSION

Coriolis effects (CE) are conceived to arise from cross-coupled angular accelerations sensed by the different semicircular canals in response to an inclination of the head while the head is undergoing a passive rotation about an axis not aligned with the head tilt. A mathematical analysis has been given in terms of the physical properties of a spinning top (Groen, 1961) or in terms of Coriolis forces (Guedry & Montague, 1961; Peters, 1969). A more vivid and non-mathematical appraisal has been proposed by Melvill Jones (1970). Subjectively apparent tilt is composed of a sensation of tilt about an axis orthogonal to the rotation axis and the head tilt axis due to the momentum excess in the horizontal canal and a sensation of increased rotation about the original axis that is due to the sud-

den stimulation of the vertical canal. Thus, for example when a subject, who has equilibrated to a constant velocity rotation to the right about an earth-vertical axis bends his head to the right shoulder he will experience a tilting backward and a renewed sensation of rotation to the right. The magnitude of the perceived tilt, however is not directly predictable from canal mechanics, because of conflicting otolith information.

Pseudo-Coriolis effects (PCE) and motion sickness may be elicited by equally bending the head out of the axis of rotation of a moving visual surround that induces the compelling illusion of self-rotation (circularvection, CV). With the visual surround moving to the left and CV developing to the right, a head tilt to the right shoulder will result in a perception of tilting backward and ongoing rotation to the right. PCE, in contrast to CE, require the sensation of self-rotation and, by definition, occur without actual rotation of the body. The neurophysiological mechanisms through which PCE arise are not yet clearly understood. Similarities in time course, relation to stimulus velocity and dependence on stimulus area and stimulus location within the visual field[1] may support the assumption that PCE and CV are based on a common process of excitation. On the basis of psychophysical data, it has been argued by Brandt et al. (1971) that convergence of visual information into the vestibular system may provide this basis. This hypothesis is supported by evidence from a few neurophysiological experiments: optokinetic stimulation can induce a direction specific modulation of resting discharge in the vestibular nerve of the goldfish (Klinke & Schmidt, 1970) and in the medial and lateral vestibular nuclei of

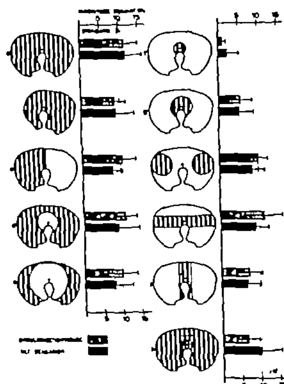


Fig. 6. Subjective velocity of circularvection and magnitude of perceived tilt in PCE in relation to area and location of stimulation within the visual field. *a*, entire visual field; *b*, monocular visual field, half of the binocular visual field, central masks 60° (*d*) and 120° (*e*); central field 30° (*f*) and 60° in diameter (*g*); two peripheral fields 60° in diameter (*h*); horizontal streak (*i*) and vertical streak (*j*) subtending 30° each. Opposite optokinetic stimulation of center (30°) and periphery of the visual field (*L*). (Means and S.D.).

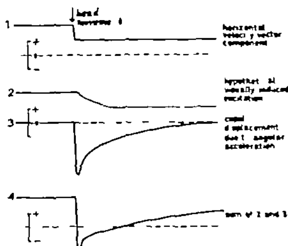


Fig. 7. Schematic drawing of the hypothetical interaction between visual input (second trace) and vestibular input (third trace) during Coriolis effects.

[1] The predominant role of the peripheral retina in dynamic spatial orientation has been discussed earlier by Brandt et al. (1973) and Dichgans & Brandt (1973).

the rabbit (Dichgans & Brandt, 1972) In the rabbit some of the neurons in the vestibular nuclei respond not only to angular accelera tion but also to exclusively visual motion stimulation. The directional specificity of these neurons is opposite for visual and vestibular stimuli. This corresponds to the natural condition in which rotation of the animal to the left is accompanied by relative surround motion to the right and vice versa. Prolonged summation and decay of frequency modulation after stimulus onset and termination roughly correspond to the time course of CV

In the case of PCE, head movements are performed in relation to the stationary body The vestibular stimulation that is provoked by the head movement, therefore, lacks cross-coupling effects from the different semicircular canals that arise from head movements during body rotation PCE seem to arise from a combination of vestibular excitation that is induced by the moving visual environment and is sustained throughout optokinetic stimulation and afferent information that is generated through the head movement itself The importance of the afferent vestibular com ... in provocation of PCE is suggested by ... fact that PCE and optokinetic motion ... ... iess up to now have not been induced in labyrinthine-defective subjects. Circular vection and shift of perceived vertical (Dichgans et al. 1972) however were elicited in all of the few patients studied thus far

PCE may be provoked up to 30 seconds after the end of the moving visual stimulus. Therefore the shifting of the direction of motion of the visual pattern over the retina induced by the head movement cannot be a necessary condition for the illusion. The visually induced excitation which, according to psychophysical and neurophysiological experiments, lasts after stimulation has ceased, is a sufficient basis for the production of PCE. A model that would explain the pseudo-Coriolis effects entirely including the surprising conformity of direction of the illusory tilt in CE and PCE cannot yet be proposed. On the basis of the mechanisms discussed above one would have predicted that the tilt should occur in opposite directions in CE and PCE.

Although it is agreed that vestibular Coriolis effects are mainly due to forces acting on the labyrinthine receptors, some evidence for participation of extralabyrinthine factors has been presented. Graybiel et al. (1961) found that CE elicited by active head movements are stronger than those induced by passive tilt of the whole body Possible influences of cervical afferences or corollary discharges (Teuber 1960) associated with the voluntary movement have been discussed.

Inhibition of Coriolis effects by visual motion information is suggested by the general observation that subjects are less susceptible to motion sickness when they are moving in relation to a visual environment that is stationary in space (Reason & Diaz, 1970). That the Slow Rotation Room, presenting a seemingly stationary visual surround is more effective in eliciting motion sickness than devices where the moving subject views the objectively stationary surround fits the same interpretation. Results presented here clearly indicate that this inhibition is direction-specific and quantitatively depends upon the relative speed of the optokinetic stimulus. Only optokinetic stimuli that move in a direction opposite to the body displacement inhibit CE. Those moving in the same direction markedly enhance CE. Coriolis effects that are elicited with the subject viewing a visual surround moving with respect to the observer may be called optokineticovestibular CE in contrast to the purely vestibular CE that are elicited with closed eyes in the dark or in the apparently stationary environment of the Slow Rotation Room. One possible explanation of this inhibition suggested in Fig. 7 is that the visual input converging upon the vestibular system (second trace of the figure) because of its long decay-time counteracts the effects of momentum excess in the horizontal canals (third trace). This explanation is supported by the observa-

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Theoretical formulations of the Coriolis stimulus (Guedry & Montague, 1961 Peters, 1969) indicate that the magnitude of the mechanical couples developed within the semicircular canal system is directly proportional to the angular velocity of rotation so long as both the rate and extent of the effective head movements are held constant. With respect to the psychophysical aspects of CE, it has been found by Meda (1952) and Guedry & Montague (1961) that the perceived tilt is linearly related to rotation velocity up to 60 / sec. We have confirmed these data and, therefore, disagree with the finding of an exponential increase that was described by Reason & Graybiel (1969). We may add new data on the relationship between the magnitude of pseudo-Coriolis effects and speed of the moving visual stimulus. Although the gain is lower for PCE, there is still a linear relation between magnitude of tilt and the velocity of the stimulus indicating to what extent the processes resemble each other that generate PCE, in the case of exclusive visual stimulation, and CE, during real body rotation The earlier saturation of PCE-magnitude at 90°–120 stimulus velocity was to be expected on the basis of the relation between stimulus velocity and velocity of perceived self rotation, as has been demonstrated by Brandt et al. (1973).

According to the data presented, it seems reasonable to separate at least three basic kinds of so-called Coriolis effects:

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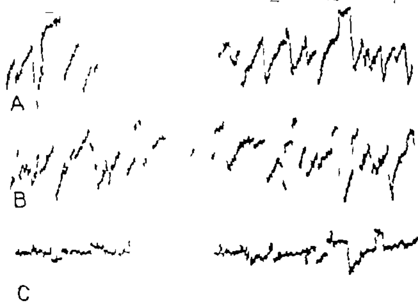


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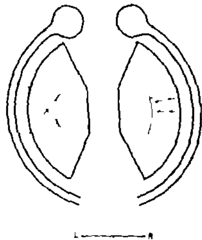


Fig 2. Schematic picture of a left-sided, vestibular neuritis. Arrows indicate the orientation of the hair cells, i.e. the direction in which they increase their discharge frequency Number of arrows symbolizes discharge frequency

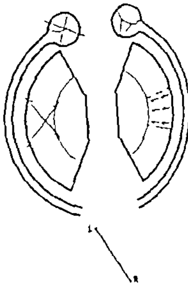


Fig. 3. ... picture of ... neuritis ... tilted to ... indicate ... direction of ... quency ... freq ... of arrows

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Acta Otolaryng 76: 349–352, 1973

INTERACTION BETWEEN THE UTRICLES AND THE HORIZONTAL SEMICIRCULAR CANALS

IV Tilting of Human Patients with Acute Unilateral Vestibular Neuritis

E. Fluur

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(Received February 28, 1973)

Abstract. Ten patients, with acute unilateral vestibular neuritis and destruction nystagmus, were placed in such a position that the utricle of the sound ear was at one time stimulated to increased activity by the g-forces, another time inhibited. In the former case, an increased nystagmus frequency was obtained, and in the latter case, an inhibition was induced which was sometimes total. Its causes are explained.

In the so-called Mach-Breuer theory (1875) it is stated that the semicircular canals and the otolith organs react on different kinds of acceleration. According to the theory, the semicircular canals are stimulated by angular and the otolith organs by linear acceleration. In a number of studies Jongkees (1954, 1966, 1967) and Philipszoon (1959, 1962) have shown that the otolith organs react on linear acceleration and gravitation, but not on angular acceleration, whereas the semicircular canals react in an entirely opposite way. However, Jongkees & Philipszoon (1962) have demonstrated that nystagmus can also be provoked by linear acceleration. These findings opened the field for new investigations at different sites with experimental animals and on human beings, in the latter, especially by using centrifugation. Graybiel et al. (1952) were unable to demonstrate nystagmus in normal human subjects exposed to increased gravitational forces induced by centrifugation. Nor could Bergstedt (1961), who used the same method, elicit any nystagmus, though he showed that linear acceleration had an influence on pre-existing nystagmus. McCabe (1964), on the other hand, observed distinct nystagmus when applying the same method. Fluur & Siegborn, (1973) have shown in the unilabyrinthectomized cat, that tilting around its longitudinal axis caused a characteristic alteration in a pre-existing spontaneous nystagmus. The cats, which had nystagmus toward the nonoperated ear, showed, during tilting toward the operated side, an increase in nystagmus frequency, and during tilting in the opposite direction an almost total inhibition of nystagmus. The author now wishes to study the problem, whether an alteration in the gravitational forces acting on the otolith organs of human subjects, with acute unilateral, labyrinthine destruction, can change the spontaneous nystagmus in the same way as in animals, thus showing that interaction between the utricle and the horizontal semicircular canals in humans is the same as in some animals.

MATERIAL AND METHOD

Ten patients with acute unilateral, vestibular neuritis and destruction nystagmus took part in the investigation. Nystagmus was recorded with the traditional electronystagmographic technique. The patients were first placed in a sitting position, with eyes open, in the dark, behind a diving mask. Spontaneous nystagmus was recorded, and the same procedure

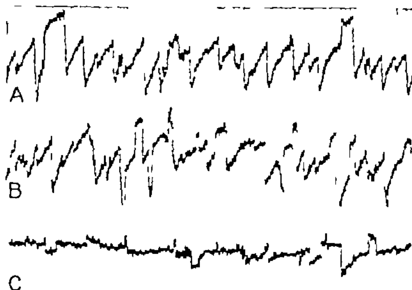


Fig. 1 Electronystagmographic recordings from a patient with acute unilateral, vestibular neuritis in the right labyrinth. A, Spontaneous nystagmus to the left, patient in sitting position. B, Nystagmus, patient in right supine position. C, Nystagmus in left supine position.

repeated, but with the patients eyes closed. After this the patients were placed in a horizontal position on their right or left side. In each position nystagmus was recorded as above with open and closed eyes.

RESULTS

6 patients, placed with their sound ear wnwards, nystagmus was totally inhibited compared with the spontaneous nystagmus at rest (Fig 1). This inhibition occurred in 4 patients within 10 sec in 2 patients 20 to 30 sec were required. In 4 patients there was no total inhibition but a distinct decrease in nystagmus frequency was observed. If on the other hand, the patients were placed with their sound ear upwards, nystagmus frequency in-

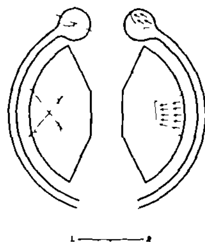


Fig. 2. Schematic picture of a left-sided, estibular neuritis. Arrows indicate the orientation of the hair cells, i.e. the direction in which they increase their discharge frequency Number of arrows symbolizes discharge frequency

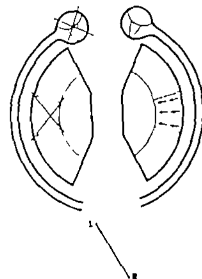


Fig 3 Schematic picture of a left-sided, vestibular neuritis; the patient is tilted toward the right. Arrows indicate the orientation of the hair cells, i.e., the direction in which they increase their discharge frequency The number of arrows symbolizes discharge frequency

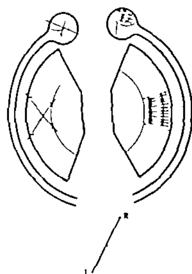


Fig. 4. Schematic picture of a left-sided, vestibular neuritis; the patient is tilted toward the left. Arrows indicate the orientation of the hair cells, i.e., the direction in which they increase their discharge frequency. Number of arrows symbolizes discharge frequency.

creased in 4 of them and remained unchanged during the recording time (1 min). In the other 6 patients there was no alteration in nystagmus frequency.

DISCUSSION

The investigation has demonstrated that an alteration in the influence of gravitation on the otolith organs can change spontaneous nystagmus in both a positive and a negative direction, precisely as in cats. This shows that the principle of interaction between the utricle and the horizontal semicircular canals seems to be the same within large areas of the animal kingdom, including homo.

Referring to earlier neurophysiological experiments concerning utricular stimulation and oculomotor reactions (Fluur & Mellström, 1970) the author will try to show by means of a few pictures how this effect is obtained. Because of labyrinthine damage the nystagmus is elicited from the horizontal, semicircular canal of the sound labyrinth (Fig. 2). Fig. 3 shows the situation where the sound ear faces downwards. This causes the otolith membrane to slide downwards, thus inhibiting the activity in the area of the utricle from which horizontal eye movements proceed. Because every activity in this region facilitates nystagmus induced from the ipsilateral, horizontal canal (Fluur & Mellström 1970), positive triggering does not occur and nystagmus is inhibited.

Fig. 4 shows the situation when the sound ear faces upwards. Here, the otolith membrane slides inwards medially. This causes the activity in the above-mentioned area to increase and, consequently, facilitates nystagmus induced from the ipsilateral horizontal semicircular canal, so that in certain cases nystagmus frequency increases.

ZUSAMMENFASSUNG

Zehn Patienten mit akuter unilateraler Vestibularisneuritis und Destruktionsnystagmus wurden in solcher Stellungen gebracht, dass der Utriculus des gesunden Ohres einmal zu erhöhter Aktivität stimuliert, ein anderes Mal inhibiert wurde. Im ersten Falle erhielt man eine Erhöhung der Geschwindigkeit des Nystagmus, und im zweiten Falle wurde eine Inhibition induziert, die mehrmals total war. Die Ursache dazu wurde erklärt.

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Acta Otolaryng 76 353–359 1973

RECONSTRUCTION OF MIDDLE EAR WITH OLD RADICAL CAVITY

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(Received January 2, 1973)

Abstract A technique of surgical reconstruction of the middle ear subjected previously to a radical mastoidectomy using only autogenous grafts, is presented. Missing bony canal walls are reconstructed with tragal cartilage and its skin lining—by using the epidermal lining of the mastoid bowl. All squamous epithelium is removed from the middle ear spaces, great care being taken to preserve the mucoperiosteum. No attempt is made to obliterate or diminish the mastoid cavity which should remain in communication with the reconstructed tympanic cavity no retroauricular drainage then being necessary Suitable ossiculoplasty usually of the columellar type, utilizing any ossicular remnants, autogenous bone or cartilage, is performed. For total or subtotal myringoplasty a large fascial graft is used. In all 22 patients thus operated on a completely healed middle ear with integrity of all grafts, was obtained. In 6 cases with a follow-up of 16 months to 4 years slightly enlarged reconstructed ear canal and a restored, sometimes less mobile tympanic membrane were found. Postoperative measurements of the canal size in two diameters: vertical and transverse, have been carried out. Functional long-term results were good in all but one case, the air–bone gap being diminished to 10–25 dB.

A radical mastoid cavity is from many points of view a highly unsatisfactory end result of ear surgery Although nowadays indications for this type of surgery are rather rare, there are still quite a number of patients with old radical mastoid cavities, the result of previous surgery For more than 10 years the trend has been to eliminate these cavities as a secondary procedure, frequently making use of variously fashioned and pedicled muscular or musculo-periosteal flaps (temporal musculoplasty Thornburn, 1961 mastoidplasty Austin & Sanabria, 1963). Of these procedures the most widely practised are now those aiming at restoration of the canal skin (Palva, 1962, Guilford, 1961). In the technique of mastoidplasty with reconstruction of the canal skin Palva (1962) and other authors stress, as an important factor complete separation of the obliterated mastoid cavity from the tympanic cavity and the ear canal. For functional rehabilitation of the patient a columellar-type ossiculoplasty is often recommended (Austin & Sanabria, 1963)

Unfortunately these muscle or musculo-periosteal flaps almost always tend to shrink and retract and, as only the canal skin is reconstructed, a marked, sometimes cavity-like retraction of the canal is often produced. Furthermore, in a certain percentage of cases complications may arise from these obliterating procedures, such as bleeding or hematoma, partial necrosis of the flap and infection, even occasional labyrinthitis (Elbrønd, 1963).

In cases with an old radical mastoid cavity few authors have so far published their experiences of complete reconstruction of *all* anatomic and functional elements of the middle ear using only autogenous grafts.

Since 1965 at the Kraków Otologic Department a technique of closed tympanoplasty if necessary with reconstruction of the canal with rigid autografts, such as bone or cartilage, has been used in the majority of patients (Sekula, 1968, 1970· Szpunar 1966). It is desirable that a reconstruction of the

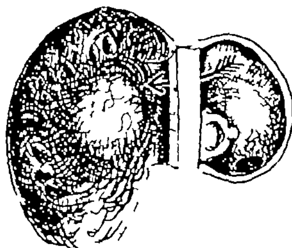


Fig. 1 Schematic representation of reconstruction of the superior bony canal wall by a tragal cartilage graft in a case after a radical mastoidectomy Note free communication between the mastoid and tympanic cavities underneath the cartilage graft (*arrow*).

canal with bone or cartilage should also be used for such extensive defects of the bony canal as in cases after a previous radical mastoidectomy In closed tympanoplasty the postoperative cavity in the mastoid process is communicated through the aditus with the tympanic cavity and the Eustachian tube. Similarly we believe that in cases with an old radical cavity reconstruction of the bony canal should not interfere with communication between this cavity and the tympanic one, to facilitate re-aeration and drainage of the mastoid cavity by the physiologic way (Fig. 1). This view is also shared by Sekula (1968). In this situation it is necessary to leave as much as possible of the mucous lining of the middle ear spaces to facilitate regrowing of this lining in the places where mucoperiosteum is deficient Thus the normal mucosal lining of any remaining mastoid cells should be spared.

In such cases it is not necessary to fill up or diminish the mastoid bowl. Aboulker et al. (1970) has already pointed out that the problem of obliterating the mastoid cavity is only secondary if reconstruction of the posterior bony canal wall has been performed. By abandoning mastoidplasty one avoids all possible complications connected with this procedure. Our experience with this type of surgery seems to support this view as we obtained prompt healing, without any complications, in all 22 cases operated on using this technique.

Full reconstruction of the middle ear with an old radical mastoid cavity is certainly one of the most difficult reconstructive operations on the middle ear In such cases only foundations of the middle ear are left" (Aboulker et al., 1970), and it is necessary to rebuild, step by step, all its anatomic and functional elements, under unfavorable conditions. Since radical mastoidectomy is usually undertaken to end long-standing middle ear suppuration, many important structures of the middle ear are missing in consequence and the remaining tissues are very often of poor biologic quality

In reconstruction of the middle ear after a previous radical operation 3 main operative stages should be distinguished.

1 Reconstruction of the posterior superior bony canal wall.
2. Restoration of the sound conduction mechanism.
3. Total reconstruction of the tympanic membrane.

Successful reconstruction of missing posterior-superior bony canal wall, with restoration of the contour of the canal, may be achieved by using either autogenous bone or cartilage. In our experience, in conformity with Mc Cleve (1969) the best material for this purpose is tragal cartilage. Its advantages are as follows. (*a*) it is easily available without a cosmetic defect, (*b*) its size is quite adequate to plug even a very large defect in the canal, (*c*) if properly wedged between the edges of the bony defect it is rigid and elastic enough to maintain the concave contour of the canal, (*d*) it easily heals in place, without any loss of tissue.

The technique of obtaining tragal cartilage

has already been described (Goodhill, 1967 McCleve 1969). We remove the entire tragal cartilage with intact perichondrium left on both sides. The cartilage is wedged between the edges of the bony defect (Fig. 1) leaving beneath the lower edge of the cartilage a gap serving as communication between the mastoid cavity and the restored tympanic cavity The canal side of the cartilage is then completely covered by the previously elevated skin lining of the mastoid bowl, which forms the canal skin.

Restoration of the sound conduction mechanism often poses difficult problems in cases with old radical cavities as usually there is a complete or nearly complete destruction of the ossicular chain. Occasionally the malleus handle, attached to a small remnant of the tympanic membrane, can be found, in which case, in the presence of an intact stapes, connection of these ossicles (malleus–stapes assembly) may be tried by an appropriately shaped piece of autogenous bone or cartilage. Usually however the sound conduction mechanism has to be reconstructed here on the columella principle (Austin & Sanabria, 1963) If an intact stapes is present the situation is evidently more favourable. It might seem that simple columellisation could be the procedure of choice here. Unfortunately the anatomic conditions found in the middle ear are rarely so favourable as to ensure efficient functioning of this mechanism, even when the facial ridge has previously been partially taken down. Therefore, in the great majority of these cases it is necessary to place an ossicular remnant, if such is found in the cavity or a suitable piece of autogenous bone or cartilage, between the stapes head and the fascial graft

If the superstructure of the stapes is lacking, as is often the case, any ossicular remnant or a L- or T-shaped strut of autogenous bone or cartilage may be used as a columella, to be covered by a fascial graft.

If the footplate is fixed Aboulker (1970) suggests removal of the footplate combined with the vein graft–stapes prosthesis, performed secondarily after successful healing of the reconstructed tympanic membrane. In our material we have no such case

Reconstruction of the tympanic membrane

In cases subjected previously to radical mastoidectomy there is almost always a total or subtotal defect of the tympanic membrane so that some method of total myringoplasty is needed. For this purpose a large graft of temporal fascia or of some other connective tissue is used.

OPERATIVE TECHNIQUE

A postauricular incision is used and the posterior wall of the canal skin is cut transversely at the level where the cavity begins. These two incisions allow very good visualisation of the mastoid bowl. If the cavity is not too big its entire epidermal lining is elevated, great care being taken not to injure the lining often adhering closely to the underlying bone. This lining is contiguous with the remaining skin of the auditory canal it is incised along the facial ridge has been partially taken down the tympanic cavity which has to be removed later Thus mobilized epidermal lining of the mastoid bowl is the most suitable material for reconstruction of the posterior-superior wall of the canal skin (Sadé, 1963)

If the mastoid bowl is a voluminous one, only an anterior part of its lining is used for this purpose, the redundant portion being thoroughly elevated and discarded. Then the bowl is revised and any cysts, diseased cells or bone are eradicated. In 3 cases we found a voluminous cholesteatomatous cyst in the posterior part of the mastoid bowl under a healthy-looking epidermal lining. If necessary the bone edges of the cavity are trimmed or smoothed. It is not advisable to remove the normal mucosal lining of mastoid cells, as from it a new lining of the whole cavity will reform.

In the next operative step any pathologic

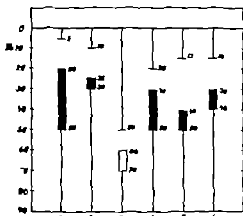


Fig 2. Individual hearing results (average speech frequencies) in 6 patients, 16 months to 4 years after total reconstruction of middle ear ■ hearing improvement □ hearing impairment. Postoperative bone conduction is indicated by a horizontal bar

material in the tympanic cavity is removed and patency of the Eustachian tube is ascertained. Evident pathology such as polypi, granulations, marked mucosal hypertrophy sometimes found under epithelial lining of the cavity is removed, greatest care being taken to preserve the mucoperiosteal layer Special attention is paid to the window region which should be cleared of all pathologic tissue Areas of typical squamous, sometimes matrix like epithelium, found at the medial tympanic wall can usually be meticulously peeled off from the mucoperiosteum. Minute defects of the periosteal layer are left alone but larger defects compromise to a great degree the end result of the operation.

Any remnants of the tympanic membrane are left in place In a case with a skin graft put in at the previous operation to seal off the tympanic cavity at least parts of it may be used as a tympanic membrane after delicate dissection from the medial tympanic wall. On the other hand, very thin membranes, passing over the window region and hypotympanum should be removed.

Then a suitable type of ossiculoplasty according to the principles previously outlined, is carried out. The condition of any ossicles found, in particular that of the stapes, is here the decisive factor

The bony canal is usually reconstructed by the use of autogenous tragal cartilage. The graft is trimmed according to the size of the defect to be filled and wedged between the edges of the bony defect. If necessary the edges are grooved for better fixation of the graft. Care is taken that the cartilage does not touch the medial wall of the attic so that free communication between the mastoid cavity and the reconstructed tympanic cavity is maintained.

The lining of the mastoid bowl, previously elevated, is placed on the canal side of cartilage to become the skin lining of the restored canal. Any defects in the lining are covered with a fascia graft tucked under their edges.

The last step of the operation is reconstruction of the tympanic membrane (total myringoplasty). The epidermal layer of any remnants of the membrane is dissected in continuity with a few millimeters of the canal skin and elevated, care being taken not to disturb the annulus or any remnants thereof At places where the annulus is destroyed, more canal skin is elevated and reflected upwards. A large temporal fascia graft is then placed so that its anterior and inferior margins lie on the annulus and are overlapped by the rim of the canal skin and, possibly by the remaining epidermal layer of the membrane. The remaining margins of the fascia graft are overlapped by the reconstructed canal skin lining If there are any defects in the skin lining, two separate fascia grafts should be used one for reconstruction of the tympanic membrane and the other for covering the defect of the canal skin lining.

Thus the reconstructed middle ear space is usually of almost normal depth. As drainage of the mastoid segment is established through the tympanic cavity to the Eustachian tube, we usually do not fill the tympanic cavity with any absorbable material. Only when it is really necessary to support the fascia graft from underneath pieces of gelfoam are placed in the middle ear space. The ear canal is lightly packed with pieces of gelfoam and strips of

Table I *Long-term anatomic results of reconstruction of the middle ear in 6 patients with an old radical cavity*

Pat. no.	Age	Condition of the cavity	Any ossicles left	Type of ossiculoplasty	Postoperative follow-up	Postoperative results
1	26	Dry Some granulations in the mastoid cells	Remnant of malleus. Loss of stapedial crura	Remnant of malleus on footplate	18 months	Dry Dimensions of ear canal 16 10 mm. Restored tympanic membrane mobile
2	18	Dry	Stapes	Simple columellisation	4 years	Dry Dimensions of ear canal 13 10 mm. Restored tympanic membrane mobile
3	35	Dry	Loss of stapedial crura	Wedge of cartilage on footplate	16 months	Dry Dimensions of ear canal 12 10 mm. Mobility of the restored tympanic membrane poor
4	34	Dry Some granulations under the lining in the window region	Remnant of incus. Loss of stapedial crura	Remnant of incus on footplate	2½ years	Dry Dimensions of ear canal 15 19 mm. Restored tympanic membrane mobile
5	36	Dry Previous tympanoplastic skin flap covers the medial wall of the middle ear	Loss of stapedial crura	Wedge of cartilage on footplate	2½ years	Dry Dimensions of ear canal 17 14 mm. Restored tympanic membrane of diminished mobility
6	23	Dry Small cholesteatoma under the lining	Remnant of malleus. Loss of stapedial crura	Wedge of cartilage on footplate	16 months	Dry Dimensions of ear canal 14 11 mm. Restored tympanic membrane mobile

gauze The postauricular incision is tightly closed.

Indications and contra-indications for this type of surgery

A typical indication for reconstruction of the middle ear after previous radical mastoidectomy exists when the patient has undergone a radical operation on the contralateral as well or when the hearing loss of the other unoperated ear is approximately at the same level If hearing of the other ear is normal or only slightly depressed an operation may still be indicated, especially if the patient has evident troubles with the ear operated on (postauricular fistula, intermittent discharge, vertigo etc.). On the other hand deafness or poor residual hearing in the contralateral ear is a definite contra-indication for reconstruction.

Before it is decided to operate on a patient with an old mastoid cavity the following conditions have to be fulfilled.

1 The ear to be operated on must have a fairly good inner ear function.

2 The patient should not be over 60 years of age.

3 The ear must be dry and free of infection for at least 3 months before the operation. Very scanty mucoid secretion with repeatedly negative bacteriologic examinations does not seem to be a definite contra-indication. On the other hand in a case showing extensive pathology in the cavity combined with an infection that cannot be controlled by any antibiotics, it is usually necessary first to perform a revision operation with the sole aim of eradicating the pathology in the middle ear and in the mastoid then to proceed to secondary reconstruction.

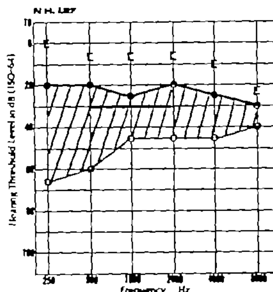


Fig 3 Postoperative hearing result, 16 months after total middle ear reconstruction in a 26-year-old woman, subjected previously to a radical mastoidectomy — preoperative air conduction curve — postoperative air conduction curve [[[, bone conduction.

RESULTS

We started this type of surgery in 1968 and have performed the technique described in 22 patients. In all patients thus operated on we have noticed rapid healing and integrity of all grafts. In the great majority of cases the ear canal has postoperatively been of a nearly normal or slightly deformed contour and its size either normal or somewhat enlarged This has been substantiated by postoperative measurements of the canal size in two diameters: vertical and transverse. In all cases the tympanic membrane has been restored, showing in 2 cases a small dehiscence and in 3 cases, markedly diminished mobility Audiometric examinations, carried out 2–8 months after the operation showed in almost all patients hearing improvement, ranging from moderate (ca. 10 dB) to very satisfactory one (30 dB)

Long-term results

Six patients have a follow-up of 16 months to 4 years. Their postoperative anatomic results, including quantitative data regarding the postoperative canal size, are presented in Table I, where details of pathology found in the middle ear at operation, and variations in ossiculoplasty are also given.

Functional postoperative results in these 6 patients are shown in Fig. 2. As can be seen, there is a more or less marked hearing improvement in 5 patients, the air–bone gap attaining values of 10–25 dB In 4 patients a serviceable hearing level (30 dB) has been reached.

The following case is an example of this type of surgery A 26-year-old woman (patient no 1 in Table I) underwent a right radical mastoidectomy 5 years before the reconstructive operation, which was performed 18 months ago As a part of the malleus was found at the operation and the stapes superstructure was absent, a wedge formed from the malleus was put on the footplate to contact the fascial graft. The defect of bony canal wall was reconstructed with tragal cartilage. The details of surgery in this case are given in Table I, and the functional result in Fig. 3.

ZUSAMMENFASSUNG

Eine neue Technik der Rekonstruktion des Mittelohres nach vorheriger Radikaloperation, anschliesslich unter Benutzung der Autotransplantaten, ist dargestellt. Fehlende Wände des Knochengehörganges werden mit Tragusknorpel rekonstruiert, der Hautgehörgang — mit der Epithelschicht der Radikalhöhle. Die Paukenhöhlengegend wird gründlich deepithelisiert, ohne die mukoperiostealen Schichte zu beschädigen. Die belassene Mastoidhöhle wird durch die Weichteile bedeckt, kommuniziert aber unter dem Tragusknorpeltransplantat mit der rekonstruierten Paukenhöhle. Eine Obliteration bzw Verkleinerung der Mastoidhöhle wird nicht angestrebt. Rekonstruktion des Schalleitungsapparates, meistens vom kolumellaren Typus, wird möglichst mit Gehörknöchelchenresten oder mit entsprechenden Knochen bzw. Knorpelautotransplantaten durchgeführt. Dann folgt totale oder subtotale Myringoplastik unter Benutzung eines grossen Temporalfaszielappens.

Bei allen 22 Patienten, die auf diese Weise operiert worden sind heilte das Mittelohr nach der Operation schnell, und alle Transplantate blieben leben. Bei 6 Patienten war die Observationsfrist länger als 16 Monate. In diesen Fällen sah der rekonstruierte Gehörgang normal aus oder war ein wenig weiter als üblich und das rekonstruierte Trommelfell war er

halten. Bei allen Fällen hat man Messungen der Weite des postoperativen Gehörganges durchgeführt. Die funktionellen Resultate nach der Rekonstruktion waren gut, manchmal sehr gut, mit Ausnahme von einem Fall, der keine Gehörbesserung aufwies; die audiometrische Knochen-Luft-Differenz (air-bone gap) betrug 10–25 dB.

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Acta Otolaryng 76 360–365 1973

HYDRON GEL IMPLANTS IN VOCAL CORDS

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(Received February 12, 1973)

Abstract. A new method for the adjustment of insufficient vocal cord was described. A Hydron rod was implanted while applying total anesthetization in a direct laryngoscopy according to Kleinsasser The implant was introduced under an operational microscope into a transverse incision of the vocal mucosa in front of the insertion of the processus vocalis. A cylindrical implant made from dry Hydron gel was used which swells in the tissue within a short time. The result of the operation performed successfully on 10 patients afforded an improvement in the quality of the voice as demonstrated by sound analysis, and also in a considerable decrease in the subjective trouble, especially disappearance of voice fatigue

The insufficient closure of the glottis due to various causes has to date been cured by injections of various liquid or semi-liquid substances into the larynx. Luchsinger & Arnold (1959) used paraffin. Arnold also used ground nasal septum cartilage, bovine bone powder tantalum oxide and Teflon or silicone particles with glycerine (Arnold 1955 1961 1962, 1963) Goff (1960) used a bovine bone paste, Lewy (1963 1964) used Teflon and tantalum, and Rubin (1965 1966 1968) applied Teflon and silicone in emulsion.

Generally speaking, however the organic particles have the disadvantage of being absorbed after some time the particles of plastics become surrounded by giant-cell granulomas, or they may migrate, sometimes penetrating into the vascular or lymphatic system, they can also have carcinogenic effects (Boedts et al. 1967 Kirschner & Svoboda 1966 Northdurft, 1955 Oppenheimer et al., 1958).

In this communication we would like to describe a new operational method for vocal cord adjustment in the case of insufficient closure of the glottis.

The method consists in that a shaped implant of hydrophilic gel, with advantage dried to a glassy hard state is introduced into the vocal cord. When in tissue the implant quickly swells to become a highly elastic soft matter

The first operation was carried out on a 21 year-old patient suffering from bilateral atrophy of the vocal muscle Since his childhood the patient had been speaking in a hoarse veiled voice in spite of repeated vocal re-education at the Clinic of Phoniatry in Prague (Head. Prof Dr Seeman) no improvement of the voice could be observed The patient had a very short phonation time (8 sec), the vocal interval being only four tones. The patient experienced a great vocal fatigue.

In the operation a rod-shaped gel (10 mm long, 1 mm dia.) was used ground on both sides to form a conical tip

Under total endotracheal anesthesia, in direct laryngoscopy after Kleinsasser and under an operational microscope, the mucosa of the upper surface of the left vocal cord was subjected to incision approx. 2 mm long at some distance from the insertion of processus voca

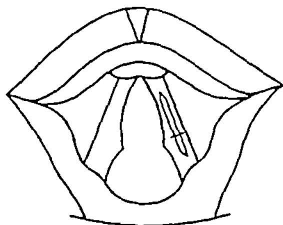


Fig. 1. Schematic view of the transverse incision and the final position of the implant.

lis. The sharp tip of the implant was then slid under the mucosa of the upper surface of the vocal cord, as near as possible to its free edge (Fig. 1).

The three-dimensional hydrophilic gel is a substance developed in Czechoslovakia for medical purposes, and known under the commercial name of Hydron. It is obtained by polymerization of the glycol esters of methacrylic acid as a continuous three-dimensional macromolecular network. By choosing specific conditions of synthesis, the structure of this substance was intentionally made such as to allow of its long-term deposition in the living tissue.

The substance was deliberately not modelled on biopolymers, so that it would not be damaged in the living tissue by enzymatic processes, and the products of its degradation (if any) would not produce immunological reactions. Despite their basically different chemical structures, these compounds have a number of properties close to those of the living tissue, from both physicochemical and mechanical points of view. A standard, structurally simplest type of this gel has been applied both experimentally and in clinical practice for more than 15 years, and its compatibility has been proved for many types of tissue (La Guerre et al., 1968 Hubáček et al., 1966 Kliment et al., 1968 Kočvara et al., 1968 Krejčí et al. 1970 Křístek et al. 1966 Levowitz, 1969 Šístek et al., 1968 Warren et al., 1967 Wichterle & Lím, 1960).

Mechanical properties of this substance (elasticity strength) and swelling can be influenced by various ratios of the initial compounds and conditions of polymerization. The three-dimensional cross-linked gels are completely insoluble in all known solvents and resist acids, bases and enzymes. The consistency of the gel can be varied within a very broad range, from the amoeba-like softest substances to glassy polymers.

One advantage of the hydrophilic gel is that it can be easily sterilized by boiling or keeping in an autoclave at 120 C, or by subjecting it to dry heat treatment at 140 C.

Permeability of the neutral hydrophilic gel for water and water-soluble compounds makes this substance basically different from the conventional plastics. A gel implanted into organism allows diffusion of water and water soluble low molecular-weight compounds. In this way the implant participates—although to a very small degree—in the metabolism of the surrounding tissue through the mass transport.

After some time a fine fibrous encapsulation of implants without any important cellular reaction takes place in the tissues.

To obtain a better penetration of the implant into the vocal cord, if the latter is considerably scarred, we made operational tools according to our own design which enabled us to obtain a submucous tunnel in the vocal cord.

The implant in the vocal cord swells within a few minutes, and becomes soft and supple. The increase in volume and similarity between the elastic properties of the implant and those of the surrounding tissue provide for the fixation of the implant.

A comparison of Figs. 2 and 3 shows a change in the shape of the vocal cord, especially the smoothing of its edge after implantation. Its mass was increased, which improved the closure of the glottis.



Fig. 2. Appearance of the vocal cord before operation.



Fig. 3. Appearance of the vocal cord immediately after operation.

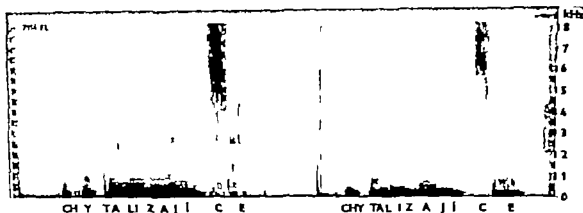


Fig. 4. Sonagram of the written text before and after operation.

For 10 days after implantation, PNC and STM were applied in injections in usual doses. The vocal cord was soaked for 2 days only and returned almost to normal within 3–4 days. Immediately after the operation the voice was more sonorous, a little deeper and slightly breathy. The patient did not have to rest his voice and there were practically no restrictions in his speech.

A week after the implantation the laryngoscopic finding showed that the spindle-like gap between the vocal cords had been considerably reduced on the side of the implant, where the vocal cord reached practically to the central line. The voice was also much better as can be seen from the voice analyses carried out before the operation and a week after. The phonation time became longer (16 sec) i.e. was doubled.

The patient himself declared that he spoke much better and with much more ease than before the operation, that the effort while speaking was much less, and that he did not feel voice fatigue. The sonagram (Fig. 4) shows before operation a pronounced breathy rustle up to 4 000–4 500 Hz; in the right hand part, after operation, the rustle has disappeared. Fig. 5 the left-hand part of the sonagram (before operation) shows a breathy rustle in the main formants of the sound á.

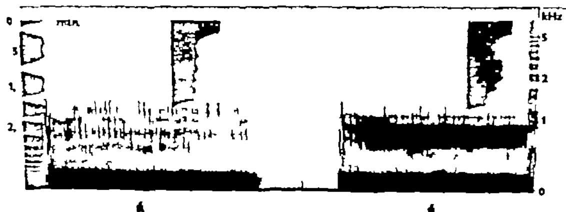


Fig. 5. Sonagram of prolonged phonation of the sound á before and after operation.



Fig. 2. Appearance of the vocal cord before operation



Fig. 3. Appearance of the vocal cord immediately after operation.

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Acta Otolaryng 76. 366–371 1973

VIDIAN NEURECTOMY IN VASOMOTOR RHINITIS

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(Received November 7 1972)

Abstract Vidian neurectomy is the recommended surgical treatment for vasomotor rhinitis resistant to conservative therapy 16 cases have been operated on over a period of 9 years. Only in one case did we not encounter complete success and in 2 cases we had relapses after 2 years on the unoperated side of the nose.

At the ENT Department of Zagreb 16 vidian resections have been performed since 1964 A comparatively smaller number of such operative procedures over a period of 9 years is the result of our stricter indications in the surgery of vasomotor rhinitis. Furthermore with the indications, we hope to discover the es- ~ of pathophysiological changes following idian resection.

AUTONOMIC INNERVATION OF NASAL MUCOSA

Tschalussow demonstrated the existence of vasomotor fibers in the nasal structure for the first time in 1913 By applying electrical stimulation he showed that vasoconstrictor fibers entered the cervical sympathetic chain, and vasodilator fibers the vidian nerve when he applied nicotine to the sphenopalatine ganglion, the vasodilator response was replaced by vasoconstriction. Blier (1930) confirmed Tschalussow s finding that vasoconstrictor fibers were present not only in the posterior nasal nerve of the dog but also in the vidian nerve, and he demonstrated that vasoconstriction disappeared after superior cervical ganglionectomy and degeneration of postganglionic fibers. Except in the vidian nerve, cholinergic fibers were found in the ethmoidal nerve and to a lesser extent in the maxillary nerve (Nomura & Matsuura, 1972). However it appears that most of cholinergic fibers lie in the vidian nerve and that it innervates the most active areas of the nasal mucosa of the lower and middle turbinate as well as the corresponding part of the septal mucosa.

The cholinergic fibers terminate mainly in the nasal glands, the arteries, and the arterioles, and to a lesser extent in the sinusoids. There were no cholinergic fibers running to or terminating at the ciliated epithelium including the goblet cells.

The vascular nasal system is richly provided with sympathetic fibers while the nasal glands are almost devoid of adrenergic nerve fibers and only occasionally are they seen near the glands. In this way the cholinergic innervation is predominant in the functioning of the nasal glands while adrenergic innervation is predominant in the functioning of the vascular system of the nasal mucosa.

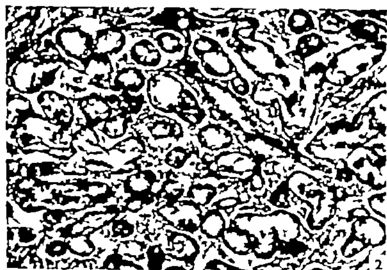
The effect of the exclusion of the parasympathetic nerves and the sensibility of the nasal mucosa have been studied in our cases of vasomotor rhinitis which had been subjected to vidian neurectomy

FINDINGS

As early as 1926 Sewall described a transantral approach to the vidian nerve in cadavers but



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Figs 1 2 3 Mucous glands of nasal mucosa before Vidian neurectomy

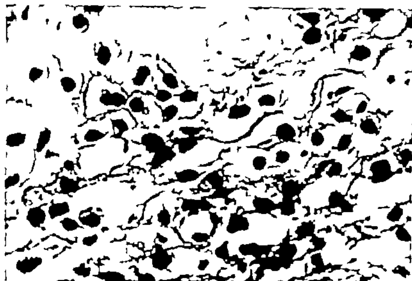


Fig 4 Eosinophils of nasal mucosa before vidian neurectomy

did not report applying it in a living patient (1937). His perfect technique was quite similar to our approach today Golding Wood (1962) recommended his technique of neurectomy of the greater superficial petrosal and vidian nerve in stubborn cases of vasomotor rhinitis in order to ablate the parasympathetic nerve

In our clinic we began to apply vidian neurectomy in 1963 Our modification of this approach was described in *International Rhinology* 1965 (Krajina & Kosoković, 1965) Until now we have had only 16 cases.

We believe that every case of vasomotor rhinitis constitutes a syndrome of the most varying aetiology For this reason a thorough analysis is necessary in order to ascertain if possible, the true aetiological factors. Following this, we use conservative treatment either locally or generally Only in cases where conservative treatment is unsuccessful and where the subjective symptoms of the patient are very marked, do we have recourse to the surgical treatment of vasomotor rhinitis.

In no case was the internal maxillary artery tied, as the posterior wall of the maxillary sinus was removed medially and superiorly where the ganglion was easily found just above the entrance of the sphenopalatine artery This structure can be easily isolated and has the shape of the lens. The lateral sensitive branch of the ganglion is not touched and the posterior branch which is actually the vidian nerve is divided. In our first cases we carried out limited coagulation of a part of the ganglion. In none of our cases were any complications encountered during or after operation. The results were excellent except for the one case where the psychosomatic background was very marked.

In all 16 cases vidian neurectomy was made unilaterally on the side of the more marked symptoms. In 2 cases we had relapses after 2 *years but on the unoperated side of the nose.*

*Golding Wood (1962) assumes that the re*flex arc for vasomotor symptoms could be via autonomic nerve fibers. Cutting the parasympathetic fibers on one side severs the ipsilateral efferent supply and reduces by half the afferent supply and a bilateral effect can be obtained.

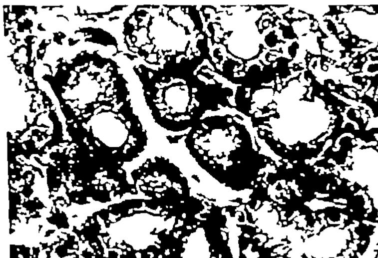
During the operation and 10 days after it, the nasal mucosa was histologically examined in order to observe the effect of vidian neurectomy In all our histological studies we have found a very pronounced histological difference of the nasal mucosa before and after vidian neurectomy While the mucous membrane of the nose before cutting of the nerve showed very numerous mucous glands in the



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6



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Figs. 5 6 7 Mucous glands of nasal mucosa after vidian neurectomy

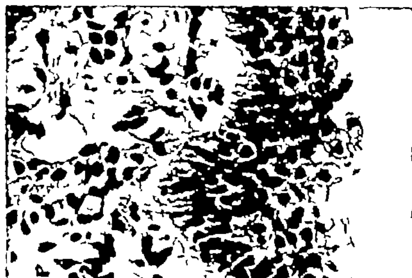


Fig. 8. Eosinophils of nasal mucosa after vidian neurectomy

hyperactive state (Figs. 1–3) after cutting the glands were decreasing in number and in a stage of normal and stabilized secretion (Figs. 5–7) Moreover eosinophils in the mucous membrane of the nose disappeared after vidian neurectomy (Figs. 4 and 8)

DISCUSSION

Vasomotor rhinitis develops basically due to autonomic dysfunction with the prevalence of the parasympathicomimetic reaction. The reasons for this can differ such as allergy emotional disturbance endocrine changes and physical agents. Parasympathetic influence has a localised characteristic because it is manifested by the parasympathic ganglion close to the organ innervated by it. Two organs, the nose and the genitals, have a special parasympathetic innervation, the richest in the body For that reason the nose has a special place in this type of autonomic dysfunction, with parasympathetic manifestations such as hydrorrhea nasalis, sneezing, and itching of the nose. The mucous glands are mostly innervated by the parasympathetic nerves, or by the vidian nerve in the nose. It seems that the secretion of the nasal mucosa in chronic vasomotor rhinitis is mostly produced by the mucous glands and for that reason the effect of neurectomy is achieved. The vidian nerve also contains some sympathetic branches from the petrosus profundus but only the parasympathetic part has its interruption in the sphenopalatine ganglion In addition the adrenergic influences come directly to the nasal mucosa through the rich vascular system of the nose.

Krajina & Poljak (1961) and Krajina et al. (1972) found experimentally a stronger sensibility of the nasal mucosa after the interruption of the sympathetic supply to the nasal mucosa. They concluded that parasympathicomimetic reaction of the nasal mucosa leads to its stronger sensibility which is reflected not only in the nasal mucosa but also on the lower respiratory tracts. Contrary to the prevalence of the sympathetic influence, this hyperactivity of the nasal mucosa is abolished.

On the basis of our histological findings we can conclude that the main effect of vidian neurectomy becomes evident upon the nasal glands and the decreased production of eosinophils as the result of the parasympathicolitic state of the nasal mucosa. In this way we can explain the diminution of the reflectory reactions of the nasal mucosa.

We consider that vidian neurectomy is the operation of choice in all cases of vasomotor rhinitis refractory to all conservative treatment.

ZUSAMMENFASSUNG

Die Vidianusneurektomie ist eine operative Therapie der vasomotorischen Rhinitis, welche mit konservativer Behandlung nicht geheilt werden konnte. In den letzten 9 Jahren wurden 16 solche Fälle operiert. Nur in einem Fall wurde kein vollkommener Erfolg erreicht. Bei zwei Patienten kam es nach zwei Jahren auf der unoperierten Seite zu einem Rückfall.

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Acta Otolaryng 76. 372-376 1973

EFFECT OF AMINOPHENAZONE, CODEINE AND DIALLYMAL ON PAIN OCCURRING IN CHILDREN AFTER ADENO-TONSILLECTOMY

A Double-blind Study

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(Received February 15 1973)

Abstract. In a double-blind trial, the effect on the postoperative pain of aminophenazone (220 mg) codeine phosphate (5 mg), diallymal (30 mg) and their combinations was studied in 159 children, about 5-7 years of age treated by adeno-tonsillectomy The efficacy of the suppositories was estimated according to the amount required. Optyl® (a combination of all the three drugs studied) suppositories were always given after two coded suppositories had been tried.

During the first 2 hours after operation the most effective treatment was the combination of aminophenazone and codeine, followed by aminophenazone combined with codeine and diallymal. 13 and 21% of the children treated with these suppositories, respectively needed two coded suppositories. Aminophenazone, diallymal or their combination were about equieffective and superior to placebo whereas codeine alone or combined with diallymal was equal to placebo. 55% of the children treated with placebo needed two coded suppositories.

On the basis of the total number of Optyl suppositories later required, the most potent treatment was the combination of all the three drugs studied 20% of the children in this group needed one Optyl suppository whereas 39% of the children treated with aminophenazone and codeine needed one Optyl suppository and 6% of them two Optyl suppositories. As distinguished from the other treatments the combination of all the three drugs differed significantly from placebo, which was the most ineffective.

This study suggests that the combination of aminophenazone codeine and diallymal relieves postoperative pain more effectively than one single drug or a combination of two drugs.

Operations such as tonsillectomy involving the superficial parts of the body produce sharp and definitely localized pain. This kind of pain is known to be satisfactorily controlled with antipyretic analgesics. Especially such children who are very much afraid of injections prefer antipyretic analgesics in the form of suppositories.

In addition to suppositories containing antipyretic analgesics there are also others in which antipyretic analgesics are combined with drugs like codeine and barbiturates. Codeine has been found to increase the potency of antipyretic analgesics (Beaver 1966). On the other hand, barbiturates do not cause analgesia without definite impairment of consciousness, and in small doses they may even increase the reaction to painful stimuli (Clutton Brock, 1961 Dundee, 1960· Keats & Beecher 1950· Neal, 1965).

For these reasons this study was designed to compare the efficacy of the combination of aminophenazone, codeine and diallymal with that of the single drugs and different drug-combinations in relieving post-adenotonsillectomy pain in children

Children and method

This study was carried out during the years 1970-1972 at the Otolaryngological Hospital of the University of Helsinki. The children studied were divided at random into eight treatment groups according to eight kinds of suppositories used. Mean age in these groups

Table I. *Age weight and sex of children in different treatment groups*

Mean ± S.E. are given

Group	No. of children	Age (years)	Boys (%)	Girls (%)	Weight (kg)
A	19	5 9 ± 0.3	50 0	50.0	20.6 ± 0.6
B	23	6.3 ± 0 4	68.4	31.6	21 4 ± 0.8
C	18	5.8 ± 0.3	72.7	27.3	20.5 ± 0.6
D	20	6.1 ± 0 4	47 1	52.9	21 7 ± 1.0
E	18	5.5 ± 0 2	76.9	23.1	20.6 ± 0.6
F	18	6.5 ± 0.3	60.0	40.0	21.8 + 0.6
G	23	6.7 ± 0.4	65 0	35.0	21.3 ± 0 6
H	20	6.2 ± 0.3	50.0	50.0	22.3 + 0.9

varied from 5.5 to 6.7 years and mean weight from 20.5 to 22.3 kg (Table I). The general condition of the children was good and, with the exception of the drugs used for anaesthesia, they received no other drugs.

Anaesthesia and operation

The children were premedicated with 1 mg/kg of pethidine and with 0.02 mg/kg of atropine intramuscularly 45 min before anaesthesia. Anaesthesia was induced with thiopental. Suxamethonium was used for intubation. The mean dose of thiopental varied from 8.0 to 10.5 mg/kg and that of suxamethonium from 1 4 to 1 8 mg/kg between different groups. Anaesthesia was maintained with nitrous oxide (67 vol%), oxygen (33 vol%) and halothane (1.5–2 vol%) via a cuffed endotracheal tube. Ventilation was spontaneous. The mean duration of anaesthesia ranged from 26 to 32 min and of the operation from 16 to 22 min between the groups studied. There were no complications in the course of anaesthesia.

In each group all the children had tonsillectomy most of them adenotomy and some of them such procedures as incision of the tympanic membranes or puncture of the maxillary sinuses (Table II). The operations were always performed between 8 a.m. and 1 p.m.

Estimation of pain and pain-relieving effect of the suppositories studied

During the first 2 hours after operation the children were observed in the recovery room and thereafter in the ward until the following morning. All nurses in the recovery room and in the ward took part in the observation of the children.

The following data were recorded on a printed sheet: the coded suppository used, indications for use, time of administration time of disappearance and possible reappearance of symptoms and possible side effects. The first suppository was inserted when the child complained of pain and/or cried and/or was restless. If the symptoms did not disappear in 30 min the child got a second suppository of the same kind. If this second suppository did not help in 30 min or if after disappearance, the symptoms recurred, the child received the Optyl® suppository (Lääketehdas Leiras, Turku). The Optyl suppository contains 220 mg of aminophenazone, 5 mg of codeine phosphate and 30 mg of diallymal. The following drugs or their combination were used.

Placebo (coded C), aminophenazone 220 mg (coded E) codeine phosphate 5 mg (coded B) diallymal 30 mg (coded F), aminophenazone 220 mg and diallymal 30 mg (coded G), codeine phosphate 5 mg and diallymal 30 mg (coded A), aminophenazone 220 mg, codeine phosphate 5 mg and diallymal 30 mg (coded D). The drugs were mixed with Witepsol W/45 mass which is a combination of glycerol esters of myristic and lauric acids. Placebo suppository contained only Witepsol W/45 mass. The shape, size and colour of all the suppositories were identical.

The trial was double-blind and the results were analysed before the code was broken. Student's *t*-test was used for statistical analysis of the results.

RESULTS

Sixteen percent of the children studied did not need any suppositories during the first 2 hours after operation in the recovery room. All children, however needed suppositories at *some stage on the day of operation*. Fig. 1 shows the need for different suppositories in

Table II *Distribution of the type of operation in different treatment groups*
Numbers of children are given in parentheses

Type of operation	Group A (19)	Group B (23)	Group C (18)	Group D (20)	Group E (18)	Group F (18)	Group G (23)	Group H (20)
Tonsillectomy	19	23	18	20	18	18	23	20
Tonsillectomy + adenotomy	15	19	14	15	15	14	20	18
Other[a]	5	3	3	2	4	5	3	5

[a] In most cases other mean incision of tympanic membranes and/or puncture of maxillary sinuses.

the recovery room. The most effective treatment was the combination of aminophenazone and codeine (H) followed by aminophenazone combined with codeine and diallymal (D). 13 and 21% of the children in these groups, respectively needed two coded suppositories. The H and D suppositories differed statistically significantly from placebo (C) (H, $p<0.01$ D $p<0.05$) but not from one another. Aminophenazone (E) diallymal (F) or their combination (G) were about equally effective. 33 31 and 26% of the children in these groups, respectively needed two coded suppositories. The least effective treatments were codeine (B) alone or in combination with diallymal (A). In these groups, as in the placebo group (C) about 55% of the children needed two coded suppositories. 6–7% of the children treated with the suppositories B, E, F and H and 15% of those treated with A needed one Optyl suppository.

Fig. 2 shows the total need of Optyl suppositories. None of the children studied needed more than two Optyl suppositories. The most effective treatment was the same combination of aminophenazone, codeine and diallymal (D) as is the Optyl suppository. D suppository differed statistically significantly ($p<0.01$) from placebo (C) the least effective treatment. 50% of the children in the placebo group needed one and 17% two Optyl suppositories. The other suppositories showed no statistically significant difference either from placebo or from one another.

Table III shows the time to the onset of action and the duration of action using the different suppositories. Most of the suppositories used relieved the symptoms in 11 to 20 min. Among the first coded suppositories diallymal (F) and aminophenazone (E) were the most rapid-acting (13 min) and codeine (B) the most slow-acting (18 min). Diallymal and codeine differed statistically significantly from each other ($p<0.05$). Of the coded suppositories given the second time, diallymal (F) was the most rapid-acting (11 min), but now the most slow-acting suppository was the combination of aminophenazone, codeine and

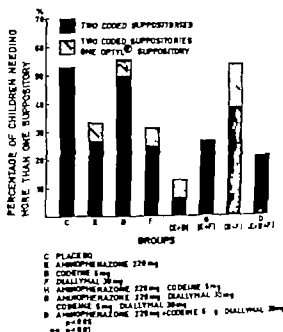


Fig. 1 Number of different suppositories required during first 2 hours after operation. There were from 13 to 19 children in the groups.

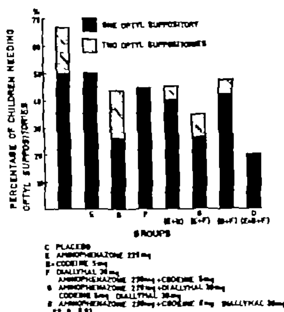


Fig 2. Number of Optyl® suppositories required on day of operation in different groups. There were from 18 to 23 children in the groups.

Table III *Time to the onset of action and duration of action of different suppositories (supp)*

Means ± S.E. are given. Numbers of children are given in parentheses

Group	Drug (mg)	Time to the onset of action (min) 1st supp.	2nd supp.	Duration of action (min) 1st supp.
C	Placebo	14 ± 2	16 ± 2	77 ± 19
E	Aminophenazone 220	13 ± 2	17 ± 2	185 ± 22
B	Codeine 5	18 ± 2	17 ± 3	173 ± 39
F	Diallymal 30	13 ± 1	11 ± 3	141 ± 29
H	Aminophenazone 220 + Codeine 5	18 ± 2	16 ± 4	146 ± 30
G	Aminophenazone 220 + Diallymal 30	14 ± 1	16 ± 3	166 ± 30
A	Codeine 5 + Diallymal 30	17 ± 2	13 ± 2	104 ± 36
D	Aminophenazone 220 + Codeine 5 + Diallymal 30	14 ± 2	20 ± 3	201 ± 57

diallymal (D) (20 min) This combination differed statistically significantly from diallymal (F) alone and from the combination of diallymal and codeine (A) ($p < 0.05$ in both cases) The combination of aminophenazone, codeine and diallymal (D) was the most long-acting (201 min) whereas placebo (C) was the most short-acting (77 min). These and the other suppositories studied did not, however differ statistically significantly from one another

The only side-effect noticed was vomiting, which was fairly rare, especially after the recovery room period in the ward. Vomiting occurred in the recovery room in 18% of the children treated with placebo (C) and in 5–6% of those receiving the combination of aminophenazone, codeine and diallymal (D) or aminophenazone and diallymal combined (G); in the ward only 11% of the children treated with codeine + diallymal (A) vomited.

DISCUSSION

The present results show that, during the first 2 hours after adeno-tonsillectomy in children aminophenazone, codeine, and diallymal, when used alone, did not relieve pain as effectively as the combination of all three drugs. Aminophenazone and diallymal were equieffective and superior to placebo They did not, however differ statistically significantly from placebo.

Diallymal, an intermediate acting barbiturate, was used in a dose of 30 mg for children weighing about 20 kg. This is about 1/3–1/4 of the hypnotic dose of 100 mg for adults and was thus a hypnotic dose for the children studied, too. The fairly good potency of diallymal to suppress the symptoms in the recovery room seems to be due to its sleep-producing action, especially when the effects of the general anaesthesia are still present. This result is in good agreement with that obtained by Keats & Beecher (1950), who found that hypnotic doses of pentobarbital intravenously relieved postoperative pain in 50% of patients compared with 20% of those treated with saline or with morphine

It is not surprising that codeine alone had

practically no effect since it was not used in an analgesic dose but only in a cough reflex suppressing dose. It did, however increase the analgesic effect of aminophenazone considerably In this case the effect of codeine was very important because aminophenazone in a dose of 220 mg was maximal for the children studied. Unlike codeine diallymal did not modify the analgesic effect of aminophenazone

Judging by the need for Optyl suppositories on the day of operation the most potent suppository was the combination of aminophenazone codeine and diallymal. As opposed to the results obtained in the recovery room, the combination of aminophenazone and codeine was now inferior to aminophenazone combined with codeine and diallymal and did not statistically significantly differ from placebo as did the combination of all the three drugs studied. This result supports the view that, after such operations as adeno-tonsillectomy in children, barbiturates are valuable in addition to analgesic drugs. One reason for this may be the sedative and hypnotic effect of barbiturates especially when the effect of the general anaesthesia has to a great extent worn off.

Analgesic combinations are used to achieve better analgesia than can be obtained with a single drug and to avoid side effects by reducing the dose of any analgesic used separately The present study showed that the combination of aminophenazone codeine and diallymal meets all the above requirements, it is more effective and causes no more side effects than the single drugs or the combination of two drugs.

ZUSAMMENFASSUNG

In einem Doppelblindversuch wurde die Wirkung von Aminophenazon (220 mg), Kodeinphosphat (5 mg), Diallymal (30 mg) und ihren Kombinationen auf postoperative Schmerzen bei 159 Kindern im Alter von etwa 5–7 Jahren, die aufgrund von Adeno-Tonsillektomie behandelt worden waren, untersucht. Die Wirkungskraft der Suppositorien wurde jeweils nach ihrer erforderlichen Dosierung beurteilt Optyl® Suppositorien, eine Kombination der drei untersuchten Präparate, wurden stets dann verabreicht, wenn zuerst zwei geschlüsselte Suppositorien benutzt worden waren.

Während der zwei ersten Stunden nach der Operation bestand die erfolgreichste Behandlung aus einer Kombination von Aminophenazon und Kodein, die fortgesetzt wurde mit einer Kombination von Aminophenazon, Kodein und Diallymal. Jeweils 13 und 21 der Kinder die mit diesen Suppositorien behandelt wurden, brauchten zwei geschlüsselte Suppositorien. Aminophenazon und Diallymal oder ihre Zusammensetzung waren etwa gleich Placebo oder waren ihm überlegen, während Kodein, allein oder in Kombination mit Diallymal, dem Placebo entsprach. Von den Kindern die mit dem Placebo behandelt worden waren, brauchten 55% zwei geschlüsselte Suppositorien.

Aufgrund der gesamten Anzahl von Optyl-Suppositorien, die später erforderlich waren, konnte festgestellt werden, dass eine Zusammensetzung der drei untersuchten Präparate am wirksamsten war Von den Kindern dieser Gruppe brauchten 20% ein Optyl-Suppositorium während von den Kindern, die mit Aminophenazon und Kodein behandelt wurden, 39% ein Optyl-Suppositorium und 6% zwei Optyl-Suppositorien brauchten. Im Gegensatz zu den übrigen Behandlungen unterschied sich eine Kombination aller drei Präparate deutlich vom Placebo, das die geringste Wirkung hatte.

Diese Untersuchung lässt vermuten dass eine Kombination von Aminophenazon, Kodein und Diallymal postoperative Schmerzen wirkungsvoller lindert als ein einziges Präparat oder eine Kombination zweier Präparate.

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Acta Otolaryng 76 377–380 1973

A CASE OF CONDITIONED FEAR IN A TWO-YEAR-OLD BOY AFTER TONSILLECTOMY

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(Received April 6, 1973)

Abstract Some behavioural sequelae after tonsillectomy in a two-year-old boy are described. It is made an essential point that the pre-verbal or pre-logical child (0–5 yrs) is especially vulnerable to hospital practices. The behavioural sequelae observed in this patient are interpreted and treated according to learning theory in contrast to the global psychoanalytical ways of treating such phenomena. Some sort of conditioned phobic reactions are supposed to occur in most pre-verbal children during hospitalization and parents should be informed to observe the behaviour of their children after they have been discharged from hospital.

Reports of the psychic consequences of hospitalization of children are mostly published in psychiatric/psychological journals, and more often than not are concerned about verbal children, i.e. children who have already acquired a verbal language. This report is about a boy whom we may consider pre verbal or pre-logical for all practical purposes.

The patient, by the time he had to have his tonsils removed, had not acquired a fear like that mentioned in the title, nor did he show any other signs of exceptional behaviour when visiting hospitals or children's ward stations for controls before the operation.

The hospital where the tonsillectomy had to be performed, did not allow the parents of hospitalized children to have contact with their children in the postoperative phase, i.e. the first 4–5 days before discharge from the hospital. Even the process of admission was known to be a little "tough" However the father of the child, by means of his professional position was allowed to follow his child into his future "living room" in which about 15 other children of roughly the same age stayed at that time. The child was undressed by his father and put to bed and he did not see his father again until he was discharged 5 days later

The Discharge

Discharge from the hospital was not the affectionate reunion the parents had hoped for The boy was in the beginning rather apathetic, but recognized his parents and his sister Some pain and irritaion must still have been present as he did not say very much, and when he did he only had the minimum opening of his mouth to form the appropriate words. No other signs of behavioural irregularity were noticed before the time came for dinner that day

During the meal he refused to eat some of the items and his mother kindly reminded him that this was the same type of food that he had been so fond of before the hospitalization. The child then reacted by *holding his hands tightly in front of his mouth* insisting that he did not like it and did not want to eat it.

At first glance this situation does not seem to differ to any degree from similar situa-

tions in any other family where a child does not accept the ingredients of the day's dinner

This was, however only the first of a series of incidents where—as a result of annoyance, stress, fear a.o his hands were tightly held in front of his mouth while he refused to join in go with pick up undress.

This was a totally new situation for the parents. It had previously not been a part of the child's behavioural repertoire and was remarkable by the fact that the reaction of the child seemed to be so inadequate in the situation where it appeared. What is the connection between holding his hands in front of his mouth and the refusal to undress, or the same behaviour and the refusal to pick up a toy from the floor?

Soon it was observed that he refused to enter into new houses or buildings where he had not been before—and even into places where he *had* been before and where he might have spent an enjoyable time. When his parents now asked why he would not come he started crying and holding his hands tightly in front of his mouth he insisted on not entering. Gradually as the rest of the family dis- d into the house the boy followed step by step, and after some time of adaptation, enjoyed the stay

A Conditioned Reaction

As this change in behaviour and we may as well say personality came about rather abruptly and was found quite troublesome to the parents and other people having contact with the child one might speculate about the initiation of the change

The author has made the assumption that his "shutting up" had developed as a classical conditioning in which the forceful taking to the operation and the unpleasant experiences and feeling from the oral cavity have functioned as unconditioned stimuli, the keeping of his hands tightly to his mouth as an unconditioned response.

The *image* of the hospital building generalized to the new houses and places he had to enter and the image and memory of the preoperational situation generalized to every situation where now his opinions clashed with someone else's—as the conditioned stimulus, and again, the keeping of his hands tightly in front of his mouth as the conditioned response

Probably one might split up all that happened during the five days and make a list of second and third order conditioned responses. But this is not justified as we know very little of exactly what happened.

The probability of a reaction in one situation to be generalized to another is rather high by this age as we for instance know from the language development of children. "Mom" and "dad" seem at the age of 18 months to be any person bigger than himself

The two reactions, (*a*) the *fear* of houses and (*b*) the reaction of *refusal* have several of the characteristics of a classical conditioned response they were quickly established, generalized to many "similar" situations and were very difficult to extinguish.

As the parents of the child very soon reacted to his behaviour we were able to make a behaviour analysis before any unguided treatment might destroy the original traits and had made it impossible to see what determined what.

The phobic behaviours, both "the refusal reaction" and the fear of houses, were sensitive to systematic desensitization in vivo. Gradually the boy was confronted with new situations, new buildings, places and persons —and as one year had passed the child might enter into most new places and buildings and even into hospitals. But still be refused to open his mouth for inspection. This last reaction *gradually diminished by* the daily brushing of his teeth by the father and the after-inspection with a dentists mirror Two years after the tonsillectomy the last sequelae seemed to have disappeared. Yet there is still

a remarkably strong fear of being left alone in a room.

Prophylaxis?

During the last two decades many publications have appeared which deal with the theme of hospitalized children (Jessner et al. 1952 Robertson, 1956 Loomis, 1956 Plank et al. 1959· Solnit, 1960· Goldman & Bochall, 1966 Lee & Greene, 1969 and many others).

Most of them deal with the verbal child, i.e. the child 4–5 years of age who has already acquired a language through which it is possible to convey most of the necessary information about what is going to happen to him in the hospital.

The toddler however is not so well understood as he himself can give only fragmentary information about his experiences. These have to be deduced from his behaviour and translated into general psychological (most often psychoanalytical) theory In psychoanalytical terms the damage caused by hospitalization has its origin in the phenomenon of separation from mother which is supposed to be extremely harmful. This may well be so But what is really happening during hospitalization of a two-year-old child may as well be described in terms of conditioning.

It seems to be very important that some parent figure be present during a hospital stay But the procedures immediately preceding operation cannot be performed without the arousal of fear in the small child and conditional fears are probably unavoidably established. It is on this particular point that the difference between the verbal and nonverbal individual is manifested. It is realistic to be afraid of pain and it is realistic to be afraid of needles and knives, but it is "magic" to be afraid of white coats, green cloth, grey brick-buildings etc. But these fears appear as conditioned reactions in children who have no frames of reference for the situations they experience in the hospital. No matter how much a parent, nurse or doctor tries to "explain" the situation to the child, he will never succeed with a pre verbal or pre-logical child.

The phobic conditioned reactions established in the hospital may manifest themselves after shorter or longer periods in the parental home outdoors, in social relations, seeing people wearing special coloured clothes, etc. Thus every parent of children having visited a hospital for an operation (duration of stay does not seem to be critical) should be told to observe their children after the return home, not only because of bleeding risks or tissue ruptures, but because the stay at the hospital may have caused some conditioned form of behaviour which might lend itself to treatment before secondary or tertiary complications occur

ZUSAMMENFASSUNG

Es werden nach Tonsillektomie einige Folgekrankheiten in der Verhaltensweise eines zwei Jahre alten Knaben beschrieben. Es wird stark betont dass das präverbale oder prälogische Kind von 0 bis 5 Jahren gegenüber Krankenhausbehandlungen besonders anfällig ist. Die beobachteten Folgekrankheiten im Verhalten dieses Patienten wurden gedeutet und in Übereinstimmung mit der Lernpsychologie im Gegensatz zu den globalen psychoanalytischen Methoden bei der Behandlung solcher Phänomene behandelt. Es kann angenommen werden, dass bei den meisten präverbalen Kindern während des Krankenhausaufenthalts irgendeine Art von bedingten phobischen Reaktionen auftritt, und dass daher die Eltern aufgefordert werden sollten, das Benehmen ihrer Kinder nach einer Entlassung aus dem Krankenhaus zu beobachten.

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Acta Otolaryng 76 381–394 1973

MULTIPOLAR NEURONS IN THE SPIRAL GANGLION OF THE RAT

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(Received March 22, 1973)

Abstract Light and electronmicroscopical studies of the rat spiral ganglion demonstrate that small neurons which are filamentous, thinly encapsulated and contain sparse rough endoplasmic reticulum are consistently present at all ages from weanling to sexually mature. These cells are further characterized by eccentricity of the nucleus, and by the presence of dense-cored vesicles (1 200–1 400 Å in diameter) scattered in the vicinity of the Golgi complex, in the cytoplasm, or in the cell processes. These neurons comprise about 7–8% of the ganglion cells and correspond to the unmyelinated neurons described for other species. By use of serial sections, the cells have been determined to be multipolar Ultrastructurally they resemble known parasympathetic neurons. Thus, it is suggested that the small, unmyelinated spiral ganglion cells are autonomic (parasympathetic) neurons.

Results of three different investigative methods, Golgi, histochemical, and ultrastructural, have respectively indicated that a small population of multipolar autonomic or unmyelinated neurons exists in the mammalian spiral ganglion This research was undertaken to learn whether or not the various methods utilized have merely exposed different qualities of the same group of neurons.

On the basis of histochemical studies in the mouse (Ross, 1969) rat and kitten (Ross, 1971 *b*), it has been postulated that multipolar postganglionic parasympathetic neurons exist in the spiral ganglion. This assertion runs counter to the commonly accepted belief that all of the spiral ganglion cells are bipolar and sensory Yet a search of the literature shows that early anatomists (Ayers, 1893 Retzius, 1895 Cannieu 1899 Bovero 1914) described multipolar spiral ganglion cells. While some of the findings might be ascribed to the investigative procedures utilized and to artifact, Ayers original account of multipolar spiral ganglion cells in several vertebrates was based upon studies of Golgi preparations, which are less open to question.

Retzius (1895) confirmed Ayers findings and described the multipolar neurons in his Golgi preparations of the mouse as tripolar for the most part, although quadripolar cells occurred occasionally (Fig. 1). Retzius attempted to attach some meaning to these cells and thought that they might be primitive bipolar neurons. This speculation apparently met with acceptance by his contemporaries (see Kölliker 1902) with the result that the existence of multipolar neurons in the spiral ganglion as established by Ayers and Retzius has largely been forgotten or ignored.

While no investigator working at the ultrastructural level has dealt with the question of whether or not multipolar spiral ganglion cells exist, there is ample ultrastructural evidence that a small proportion of spiral ganglion cells is morphologically different from the main population in various mammals (guinea pig Suzuki et al. 1963 Nishimura et al., 1965 Thomsen, 1966 Awataguchi et al. 1967 Kellerhals et al., 1967 Reinecke, 1967 rabbit. Suzuki et al., 1963 cat: Spoendlin, 1971 1972, and human Reinecke, 1967 Kel-

This work was supported by Grants NB-07306 and NB-07473 USPHS.

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Fig. 1 In this drawing, Retzius illustrates his finding of multipolar (in this case tripolar) neurons in the spiral ganglion of the mouse. Retzius, figure 2, 1895

pieces which were freeze-dried, mounted on stubs, coated with gold–palladium and observed in a JEOL JSM U3 scanning electron microscope

OBSERVATIONS

Light microscopy

Light microscopic studies were carried out on the thick (1–2 μ) sections of Epon-embedded material stained with toluidine blue and used for orientation purposes. In these sections, small neurons (12–15 μ) of obviously different staining quality from the bulk of the spiral ganglion cells occur singly or in small clusters of two or three (Fig. 2) These cells are located within the spiral ganglion, along its peripheral border near the root of the osseous spiral lamina, or among the fibres of the intraganglionic spiral bundle.

The perikarya of these small neurons are round or ovoid, and the nuclei are eccentric. The cytoplasm of these cells stains very lightly with toluidine blue, indicating that Nissl sub-

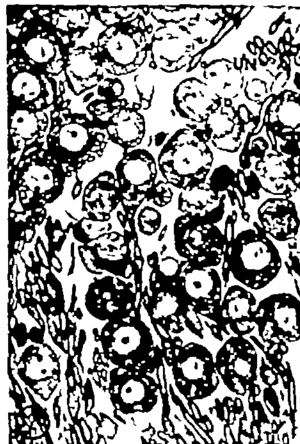


Fig. 2. Two small unmyelinated neurons (*UN*) located near the peripheral border of the spiral ganglion are shown in this photomicrograph. The cells have eccentric nuclei and cytoplasm which stains lightly with toluidine blue indicating scanty Nissl substance. 250 g male rat. ×620.

stance is sparse in contrast to that of the remaining ganglion cells which stain more darkly. Moreover the capsules of the small neurons do not take on the dark blue colour typical of myelin in this stain and thus appear to be unmyelinated.

Cell counts of the number of the myelinated and unmyelinated spiral ganglion cells were carried out in two series of thick sections, utilizing every tenth section. On the basis of these cell counts, the small unmyelinated neurons are estimated to comprise 7–8% of the spiral ganglion cells in the rat.

Transmission electron microscopy

Most of the neurons of the rat spiral ganglion are myelinated bipolar cells. The ultrastructural features of these cells are well known since the work of Rosenbluth (1962) and only a few comments are warranted here.

The round to ovoid perikarya of the bipolar cells (Fig. 3) are large (30–40 μ) and enveloped by compact myelin. The cytoplasm generally contains abundant rough endoplasmic reticulum much of which is regularly organized, giving the cells a granular appearance. In occasional bipolar cells the rough endoplasmic reticulum is less obviously organized and neurofilaments are more prominent. The nucleus is generally centrally located. The two processes are not of equal size with the central process of greater diameter than the peripheral. However both of the processes of the bipolar cells are myelinated.

Scattered within the spiral ganglion or at its periphery near the root of the osseous spiral lamina, are the cells which appear to be unmyelinated in light microscopic preparations. These cells, which were not described by Rosenbluth, will be considered in detail here.

Perikarya of these cells generally vary from 12.5–15 μ in size and are round or ovoid (Figs. 3 and 4). The plasmalemma of the small cells is densely osmiophilic and presents a scalloped surface toward the satellite cell membrane which is generally more smooth. The nucleus is eccentrically placed (Fig. 4) and contains finely granular chromatin material, which is generally more densely arranged than the chromatin in cells identified as typical, bipolar spiral ganglion cells. Some of the chromatin material is clumped in the nuclear sap and at the inner lamina of the nuclear envelope except at the nuclear pores. Typically the nucleoli are eccentrically placed in the nuclear sap.

The small neurons are further characterized by the dense packing of the cytoplasm with neurofilaments and by the relative sparseness of rough endoplasmic reticulum (Figs. 5–7). Small aggregates of polyribosomes and occasional, single sometimes branched, cisternae of rough endoplasmic reticulum are scattered



Fig. 3 Ultrastructural features of myelinated (*lower right*) and unmyelinated (*upper left*) neurons are contrasted in this electron micrograph. *C* capsule; *G* Golgi complex, *M* mitochondrion, *NSC* nucleus of satellite cell; *P* profile of a process enveloped by satellite cell *RER* rough endoplasmic reticulum *UNF* unmyelinated fibre 250 g female rat. 5 000.



Fig 4 A typical profile of an unmyelinated spiral ganglion cell is shown here *C* capsule *G* Golgi complex *N* nucleoli; *NF* neurofilaments, *NSC* nucleus of satellite cell, *NUC* nucleus of the neuron, *P* process; *RER* rough endoplasmic reticulum. 250 g female rat. × 10 000.

in the cytoplasm and comprise the sparse Nissl substance

Organized rough endoplasmic reticulum corresponding to the Nissl bodies of light microscopy is not present. Profiles of rough endoplasmic reticulum rim the outer lamina of the nuclear membrane. Additionally numerous polyribosomes and free ribosomes are associated with the outer nuclear lamina, giving this region of the cell a darkly granular appearance (Figs. 3 and 4).

The Golgi complex is elaborate and consists of several C-shaped stacks of cisternae which are situated in the perinuclear cytoplasm (Figs. 4 and 5) Numerous vesicles 1 200–1 400 Å in diameter containing dense cores, are commonly found in close proximity to the Golgi complex (Fig. 5) and are also scattered

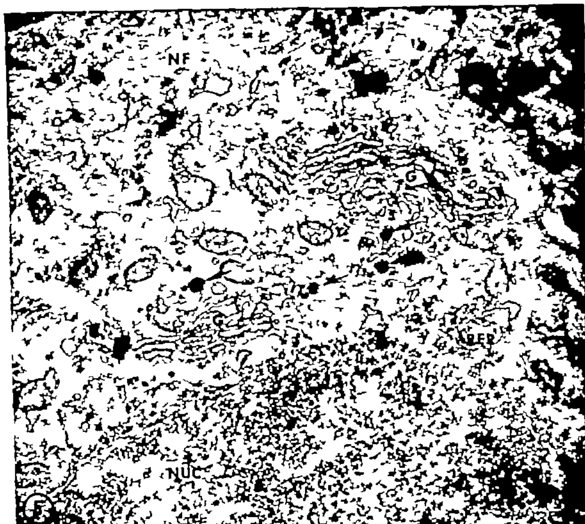


Fig. 5 The region of the Golgi complex (*G*) of an unmyelinated neuron illustrates the presence of vesicles with dense cores (*arrows*). Abundant neurofilaments (*NF*) and scanty rough endoplasmic reticulum (*RER*) occur in the field. *NUC* nucleus of the neuron. 310 g male rat. 50 000.

in the cytoplasm or in some of the cell processes (Fig. 7) The dense cores measure 900–1 000 Å in diameter Multivesicular bodies occur near the Golgi complex and scattered in the neuroplasm.

The randomly placed mitochondria, although abundant, are smaller in the unmyelinated neurons than in the typical bipolar cells (Fig. 3). Near the roots of the cell processes, mitochondria become oriented in the long direction of the processes.

The actual number of processes given off from the perikarya of these cells can be established only by a study of serial sections for most frequently only a single process continuous with a given cell body can be seen in any one ultrathin section. However occasionally two processes emerge near one end of the ovoid perikaryon, suggesting that the cells are multipolar (Fig. 6). Multipolarity of these cells was confirmed by a study of serial sections, for the cells examined in this way possessed three processes. None of the processes were enveloped in compact myelin.

It has not been possible to distinguish the dendrites from the axon on the basis of inter

nal ultrastructure. Neurofilaments, microtubules and free ribosomes occur in all processes and vesicles with dense cores occur in some. However the processes can be divided into two groups to some extent on the bases of size, configuration and ensheathment of their proximal segments.

In one group the processes arise from a broad base (2.5–4 μ) and are broad initially (1.5–2 μ) The proximal segment is usually straight and is sometimes covered by several wrappings of satellite cell membrane which terminate along it sequentially much like Schwann cell laminae near nodes of Ranvier (Figs. 7 and 8) Just distal to the last lamina of satellite cell wrapping, these processes often give off collaterals (Figs. 7 and 8). Processes of the second type tend to be small initially (1–1.5 μ) and frequently emerge from the perikaryon in a region covered by that portion of the satellite cell containing its nucleus (Fig. 3). The nucleus, in these cases, occupies the angle between the emerging process and the neuronal perikaryon. The processes are not straight but coil about close to the perikaryon. Thus, processes of this second type are often observed in cross or tangential ction enclosed within the satellite cell, and connected to the perikaryon.

Synapses have not been observed on either type of process just described. However thinly myelinated and unmyelinated fibres are always present near the perikarya and processes of the small neurons, and unmyelinated fibres have been observed winding about the more distal portions of the coiled neuronal processes.

In the rat, the small neurons are thinly encapsulated in from one (Fig. 9) to three or in very small areas, as many as five or six wrappings of satellite cell sheath. Where multiple wrappings occur the organization of the sheath usually corresponds to loose myelin (Fig. 7) However patches in which the laminae are closely apposed also occur these patches have the general configuration of semi-compact myelin Finger-like projections of the satellite cell frequently reach out to envelop unmyelinated fibres lying close to the neuronal perikaryon.

Scanning electron microscopy

The present descriptions are based upon observations of fortuitously placed cells which exhibited a bipolar or multipolar nature on a surface view In each instance the cells were checked by taking several micrographs as the specimen was rotated through 180 and tilted from −5 to +45 the maximum degrees of rotation and tilt possible with the stage utilized.

The typical bipolar cell has two processes located at nearly opposite poles of the perikaryon The central process is greater in diameter than the proximal part of the peripheral process, although the latter may become abruptly enlarged distally Perikarya of these cells are round or ovoid.

Perikarya of cells exhibiting more than two processes in the present material are ovoid (Figs. 10–12) and tend to be smaller than the perikarya of the neighboring bipolar cells. The processes of these multipolar cells are not uniform in size Two processes sometimes

Fig. 6 Portions of two unmyelinated neurons (*UN*) located near the intraganglionic spiral bundle (*IGSB*) are shown here. The cell in the centre of the field is sectioned near one pole of its ovoid perikaryon. Two processes (*arrows*) emerge near this pole the process at the right may be dividing. *TM* thinly myelinated fibres; *UNF* unmyelinated fibre 250 g female rat 4 000.

Fig. 7 Processes (*P*) of unmyelinated neurons frequently give off collaterals (*double arrows*). Four-six layers of satellite cell resembling loose myelin (*LM*) envelop the perikaryon near the process. These layers end along the process much like Schwann cell laminae near a node of Ranvier A vesicle with a dense core (*arrow*) is indicated. *NF* neurofilaments, *NUC* nucleus of the neuron *RER* rough endoplasmic reticulum. 310 g male rat. 16 000.

Fig. 8 A collateral of a process (*P*) of an unmyelinated neuron is indicated by double arrows. *NF* neurofilaments; *RER* rough endoplasmic reticulum. 310 g male rat. × 25 000.


UNF
UN
UN
TM
UNF


P
NF
7


NF
RER
8

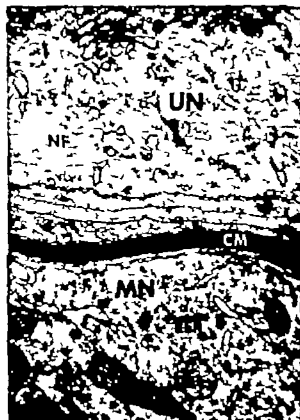


Fig. 9 The capsules of unmyelinated (*UN* upper profile) and myelinated (*MN* lower profile) neurons of the rat spiral ganglion are contrasted here. The unmyelinated neuron is encapsulated by a single layer of satellite cell (C) the myelinated neuron is en- [illegible] in nine layers of compact myelin (*CM*). *G* [illegible] complex, *M* mitochondria, *NF* neurofilaments; *RER* rough endoplasmic reticulum *arrow* a vesicle with a dense core. 310 g male rat. × 49 000.

emerge near one pole of the ovoid cell body (Figs. 11 and 12) Terminals upon the perikarya or proximal portions of the processes of these neurons have not been found.

DISCUSSION

Most of the neurons of the spiral ganglion are bipolar and myelinated and correspond to the Type I and Type II cells as defined by Rosenbluth (1962) The Type I cells are the more common in the present material for most of the bipolar cells possess abundant organized rough endoplasmic reticulum.

In the rat a small percentage (approximately 7–8%) of the neurons are not struc





Fig 10 The arrows indicate three processes of this small neuron located near the peripheral border of the spiral ganglion. 113 g male rat. × 300

Fig 11 Two processes (*arrows*) emerge near one pole of the perikaryon of this spiral ganglion cell. A third process (*double arrows*) appears to have been broken off the opposite pole during preparation of the sample 145 g male rat. × 5 000.

turally similar to either of the two types of bipolar cells but comprise a third population of spiral ganglion cells. Neurons of this third type are chiefly characterized by their thin capsules, numerous neurofilaments, scanty rough endoplasmic reticulum and eccentric nuclei. Cells of this third type which have been studied in serial sections possessed three processes. However findings obtained from scanning electron microscopy of the spiral ganglion suggest that some of the cells have four processes.

The meaning of the vesicles with dense cores commonly found in the region of the Golgi complex, or scattered in the cytoplasm or in the processes of these cells, is presently obscure. The size of the vesicles precludes their containing catecholamine for storage of amines has been associated primarily with dense-core vesicles of much smaller size (500 Å in diameter De Robertis and Pellegrino De Iraldi, 1961)

The present findings are in general agreement with those of numerous other investigators who have indicated that a group of small neurons which are highly filamentous, thinly encapsulated, and which contain sparse rough endoplasmic reticulum, exist in the spiral ganglia of many forms (Suzuki et al. 1963 Nishimura et al., 1965 Thomsen, 1966 Awataguchi et al. 1967 Kellerhals et al., 1967 Reinecke, 1967 Spoendlin, 1971 1972). These neurons, which have been called "unmyelinated" spiral ganglion cells, have also been found at the light microscopical level (Münzer 1931).

Because similar neurons were not described in the previous ultrastructural study of the rat spiral ganglion carried out by Rosenbluth (1962) it is essential to establish that they are not simply immature cells in the process of myelinization. It is necessary therefore, to compare sizes and ages of the rats used in the prior and the present studies and to define the term "mature"

In his study Rosenbluth used Mendel-Osborne rats which were said to average 150 g each and to be "mature" Although the Mendel-Osborne rats used by Rosenbluth would, as a group, be slightly lighter in weight than comparable animals of the Sprague Dawley strain used in the present study 150 g rats would not be expected to be sexually mature and adult in that sense of the word. It must be emphasized that it is only the use of the term "mature" in connection with 150 g rats that is questioned here. Whether or not achievement of sexual maturity has any bearing upon the state of maturation of spiral ganglion cells remains to be shown. Indeed, Wada (1923) has found that, at the level of the light microscope, spiral ganglion cells are of adult size and histological appearance at 20 days of age.

In the present study to settle the question of maturity spiral ganglia were obtained from rats ranging between weanling (22 g) and sex



Fig. 12. Three processes of this multipolar neuron are indicated by solid-line arrows. A possible fourth process is pointed out with a dashed-line arrow 129 g male rat. × 5 200.

ually mature animals (females, over 200 g, males over 300 g). Small neurons were found in the spiral ganglia of all the rats studied, from weanling to the sexually mature This is evidence that these cells represent a distinct type of spiral ganglion cell in the rat.

While it is true that the small neurons always appear to be unmyelinated in light *microscopical preparations, ultrastructural* studies have shown that the perikarya of the cells may be partially enveloped by more than one wrapping of satellite cell (in human spiral ganglia, Kellerhals et al. 1967 and the present results in the rat) Furthermore occasional processes of the neurons in guinea pig (Thomsen 1966) and cat (Spoendlin, 1971) are compactly myelinated. As it is generally (and admittedly arbitrarily) accepted that only those neurons with a single wrapping of satellite (or Schwann) cell are unmyelinated (see Schmitt, 1959· Rosenbluth, 1962), classification of these cells as unmyelinated may not be entirely suitable for all species. However if future research should establish that the small neurons are multipolar in other forms as in the case of the rat designation of them as ıpolar rather than as unmyelinated would more meaningful from a physiological as well as from a morphological viewpoint. All known peripherally located multipolar neurons are autonomic postganglionic cells.

As Spoendlin (1971) has already pointed out, the small, unmyelinated spiral ganglion cells cannot be postganglionic sympathetic neurons. There is no evidence from numerous fluorescence studies that adrenergic neurons occur in the spiral ganglion (Spoendlin & Lichtensteiger 1966 1967 Terayama et al., 1966, 1968 Ross, 1971 *a*) However the possibility that the cells are parasympathetic postganglionics should be considered on two grounds. 1) they correspond in distribution to acetylcholinesterase-positive neurons described as parasympathetic in the rodent (Ross, 1969 1971 *b*) and 2) ultrastructurally they resemble parasympathetic postganglionic neurons found in the rabbit otic ganglion (Dixon, 1966) or in the otic, ciliary and pterygopalatine ganglia of the golden hamster (Yoshida, 1968)

Ultrastructural studies of cranial parasympathetic postganglionic neurons are meager and, so far as we have been able to determine, the two studies quoted are the only ones existent. It is somewhat surprising that the postganglionic neurons described in these two reports are more alike than dissimilar ultrastructurally considering that they were obtained from several ganglia and from different forms. The chief characteristics of the cranial parasympathetic neurons reported upon are 1) organized rough endoplasmic reticulum is not present 2) the Nissl substance is generally sparse often uniform in distribution, and comprised of rough endoplasmic reticulum and numerous free polyribosomes; 4) dense-core vesicles occur in the region of the Golgi complex, in the cytoplasm and in some of the cell processes. Eccentricity of the nucleus was not a constant finding, but was reported for some of the rabbit otic ganglion cells.

Encapsulation of the postganglionic parasympathetic neurons of the golden hamster was not detailed by Yoshida (1968). However Dixon (1966) described the capsules of rabbit otic ganglion cells as thin, although comprised of up to five satellite cells whose processes frequently overlapped.

In the case of the present rat material, synaptic sites of the preganglionic fibres on the postganglionic neurons have been elusive. Connections of axo-axonal type between unmyelinated fibres possessing different ultrastructural features have been observed frequently in or near the intraganglionic spiral bundle (Ross, 1971 *b*) However these have been interpreted to be possible sites of influence of the parasympathetic system over adrenergic outflow In the prior studies of parasympathetic neurons alluded to above synaptic sites were dealt with adequately only in the case of the ciliary ganglion in which axosomatic synapses are abundant Yoshida (1968) suggested that the ciliary ganglion oc

cupies a unique position among parasympathetic ganglia on the basis of these numerous axosomatic synapses, and did not describe synapses in the other ganglia he studied. Dixon (1966) commented that synapses occurred in the rabbit otic ganglion, but neither described nor illustrated them. It is possible that synapses may be difficult to find in some parasympathetic ganglia where as few as two or three synapses per neuron can occur.

Processes of the small neurons have not been enveloped in compact myelin in the present rat material, in contrast to the situation in the guinea pig (Thomsen, 1966) and cat (Spoendlin, 1971) in which occasional processes are myelinated. However, the presence of myelin would not preclude the possibility that the neurons are autonomic. It has been accepted for many years that postganglionic neurons may be myelinated, and that there is considerable variation not only among species in this regard, but even from one site to another within the same individual (Huber, 1913; Kuntz, 1934; among many others).

The unmyelinated neurons of the cat spiral ganglion have been interpreted to be a variation of the bipolar neuron responsible for the afferent innervation of the outer hair cells in the organ of Corti (Spoendlin, 1971). This interpretation is based upon the fact that both the unmyelinated neurons and the afferent innervation of the outer hair cells resist retrograde degeneration in experiments in which the eighth nerve is transected. However, if the unmyelinated neurons of other forms should prove to be multipolar, some other reason for failure of these particular afferents to degenerate must be sought. In this case, the unmyelinated neurons would have resisted retrograde degeneration not because of some inherent peculiarity but simply because their axons are not extending centralward in the cochlear nerve.

The present findings, then, are in accord with the original observations of Ayers (1893) and Retzius (1895) that multipolar neurons exist in the spiral ganglion, and they suggest that what has been referred to as unmyelinated cells are the multipolar neurons. The fact that the unmyelinated spiral ganglion cells comprise a uniformly small population in diverse species speaks for a general rather than a specific function for them. Such a concept is in keeping with the theory expressed here that they are autonomic cells. From their presence and their ultrastructural resemblance to known cranial parasympathetic neurons, we may infer that a parasympathetic as well as an adrenergic influence is exerted upon structures of the inner ear.

ZUSAMMENFASSUNG

Licht- und elektronenmikroskopische Studien des Spiralganglions der Ratte zeigen, dass kleine Neuronen, die filamentös und dünn eingekapselt sind und wenig granuläres, endoplasmatisches Retikulum enthalten, durchgehend in jedem Alter von der Entwöhnung bis zur Geschlechtsreife vorhanden sind. Die Zellen sind weiterhin charakterisiert durch einen exzentrischen Nukleus und die Anwesenheit von elektronendichten („dense-core") Vesikeln (1 200–1 400 Å Durchmesser), die in der Nähe des Golgi-Apparates im Zytoplasma oder in den Zellfortsätzen verstreut sind. Diese Neuronen bilden etwa 7–8 % der Ganglienzellen und sind mit den unmyelinierten Neuronen vergleichbar, die für andere Gattungen beschrieben sind. Mit Hilfe von Serienschnitten wurden die Zellen als multipolar erkannt. In der Ultrastruktur ähneln sie bekannten parasympathischen Neuronen. Es wird daher vorgeschlagen, dass die kleinen, unmyelinierten Spiralganglienzellen autonome (parasympathische) Neurone sind.

ACKNOWLEDGMENTS

We thank Dr J. E. Hawkins, Jr, Dr E. Lauer and Dr B. Baker for their helpful comments on the manuscript.

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Acta Otolaryng 76 395–401 1973

AMPLITUDE AND LATENCY STUDIES OF THE AVERAGED AUDITORY EVOKED RESPONSES TO TONES OF DIFFERENT INTENSITIES

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(Received April 2, 1973)

Abstract "Saturation" and decline in peak amplitude of human scalp-derived averaged auditory evoked responses (AER) were regularly observed at high intensity levels of sound stimulation. The postsynaptic origin of these phenomena is emphasized. It was found that "saturation" and decline arise at relatively low sensation levels in persons with unilateral deafness (i.e. when "cross hearing" is excluded) as compared with persons having normal hearing. This suggests that the AER generating mechanism contains two relatively independent symmetrical systems which are unequally influenced by the ipsilateral and contralateral cochleae. Data are also presented (e.g. "saturation" of the AER amplitude at lower stimulation levels, different thresholds and individual changes of the early and late components of AER) which are indicative of the complex mechanism of the formation of the human AER.

The relation between the intensity of sound stimuli and the parameters of scalp-derived averaged auditory evoked responses (AER) is rather complicated. In the range of low and moderate intensities any increase in the sound pressure level causes a rise in the amplitude of AER and a corresponding reduction in the AER peak latencies (Beagley & Knight, 1967). At high intensities, the amplitude of AER remains unchanged ("saturation") or even decreases (Davis & Zerlin 1966 Butler et al., 1969 Picton et al. 1970 Kollár 1971)

It is not clear so far which parameters of the AER show a closer relation to the stimulus intensity and whether "cross hearing" may affect the results of AER amplitude and latency measurements under the conditions of monaural stimulation.

In order to eliminate the effects of "cross hearing" contralateral tonal masking has been employed by Picton et al. (1970), but this causes a general shift in the AER amplitude vs stimulus intensity curve. Thus, contralateral masking complicates the experimental conditions and may cause additional difficulties in the evaluation of the data obtained.

In the present study shifts in AER amplitude and latency related to changes in stimulus intensity were investigated in order to evaluate the effects due to "cross hearing" comparative studies were conducted in persons with normal hearing and with different interaural threshold differences.

METHODS

Twenty-five persons were studied (17 women and 8 men)—13 with normal auditory function or with moderate symmetrical hearing losses and 12 with unilateral high-graded hearing loss. In the latter group the possibility of "cross hearing" could be excluded even at intensities up to 110–120 dB SPL.

The subject quietly sat in a sound-attenuated and electrically shielded room. Ton

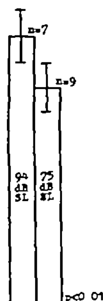


Fig. 1 Mean and standard deviation values of levels of sound stimuli at which decreases in AER peak amplitude (N1–P2) are evidenced in persons with normal hearing or symmetrical hearing losses (left column), and in persons with unilateral deafness or one-sided severe hearing loss (right column). The AER were recorded in response to 1 kHz tone bursts.

bursts of 300 msec duration with rise and decay times of 8 msec were presented monaurally by a TD-6 earphone once every 3.2 sec (in 21 cases) or 2.5 sec (in 4 cases). In 12 cases the frequency of tonal stimuli was 1 kHz, in the remaining 13 tone bursts of other frequencies (0.5 2 and 4 kHz) were also employed In some cases amplitude modulated tonal signals were presented as well. The tonal ...li were generated by a G3-18 oscillator ...n a built in electronic switch. The sound-intensity calibration was carried out by means of a Type 2007 Measuring Amplifier and a Type 4152 Artificial Ear (Brüel & Kjær) In some cases click stimuli were also used which were generated by applying 0 05 msec square pulses from a MSE-40 stimulator (Nihon Kohden) to the earphone The intensity of clicks was measured in dB comparing their main peak amplitude with the amplitude of calibrated tonal stimuli on the screen of an oscilloscope (peak equivalent SPL)

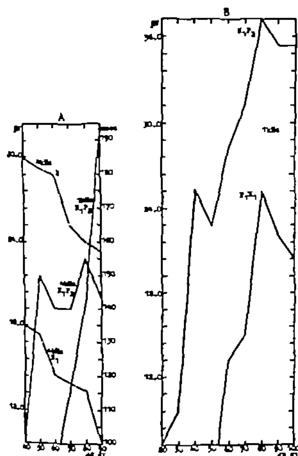
The AER were recorded from the vertex by a silver disc electrode with a diameter of 5 mm Before the application of the electrode the skin was shaved and treated with alcohol and ether The electrode was fixed with collodium The reference electrode was positioned on the ear-lobe the interelectrode resistance did not exceed 3 kΩ. The scalp-derived activity was amplified by a MB-5302 (Medicor) and an AVH 2 (Nihon Kohden) preamplifiers using filter settings of 0.5 (low limit) and 50 (high limit) Hz. For averaging, a Didac-4000 computer (Comef Intertechnique) was employed the AER were recorded photographically from the screen of its oscilloscope. From the same electrodes the EEG was simultaneously recorded by a Type 800 Mingograph (Elema-Schönander). Both the computer and the electronic switch were triggered from the stimulator In most cases, averaging was carried out over 100 presentations. The amplitude of AER was measured between the peaks of P1-N1 and N1-P2. The peak latencies of P1 N1 and P2 were determined, these peaks will be referred to as the early components of AER. The parameters of later components (N2 and P3) were also taken into account.

RESULTS

There is a comparatively simple relation between the stimulus intensity and the peak latencies of the AER components: the rise in stimulus intensity is accompanied by a gradual reduction in the latencies (Figs. 2 and 3). This phenomenon is most clearly demonstrated at sound intensities up to 20–40 dB SL, although some decrease in latency for P1 N1 and P2 was observed even at higher intensities.

The relation of the AER amplitude to sound intensity is more complicated. The phenomena of "saturation" and decline in the AER amplitude-intensity curves occur at different hearing levels. According to statistically calculated data there is a significant difference between two groups of subjects, the first including persons with normal hearing or bilateral symmetrical hearing losses, and the other group persons with unilateral deafness and unilateral high-graded hearing loss. The average value of sensation levels at which "saturation" and decline were observed was 94 dB for the first group and 75 dB for the second group (Fig 1).

The "saturation" and decline in P1–N1 and



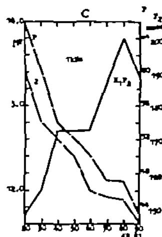


Fig. 2.

Figs. 2 and 3. Changes in peak amplitudes (P1–N1 N1–P2) and peak latencies of the main AER components (P1 N1 P2) caused by variations in sound intensity. *Abscissa:* the intensity of sound stimuli in dB SL, *ordinate* peak amplitude of AER in μV (*left*) and peak latency in msec (*right*).

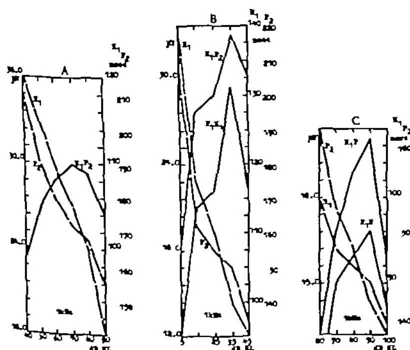


Fig. 3.

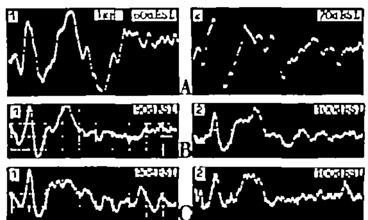


Fig 4 Difference in changes of the early and late AER components with sound intensity variations. Amplitude calibration. 10 μV time calibration: 100 msec.

N1–P2 amplitudes can be demonstrated also at moderate intensity levels (Fig. 2 B C) In both cases the decrease in peak latency is obvious (Figs. 2 and 3) With tone bursts of higher frequencies "saturation" begins earlier and is better expressed as compared to tones of lower frequencies (Fig. 2 A).

In most cases the input-output curves are similar for the amplitudes of P1–N1 and N1–P2 (Fig. 3 B C). In other cases some discrepancy between them may be observed due to the variability of P1 as compared with the more stable N1 and P2.

The changes in the amplitude of the early ponents of AER are not always accom- nied by similar changes of the later com- nents (Fig. 4) The amplitude of the later components may increase at high levels of sound intensity whereas the amplitude of the early ones is markedly reduced (Fig. 4 B C). On the other hand a rise in amplitudes of early components at lower levels of sound intensity may be accompanied by a decrease in the amplitude of the late components (Fig. 4 A) A similar discrepancy is shown in various states of the sleep-wakefulness cycle and may be observed, for instance during synchronized sleep (Fig. 6) Moreover the early components of AER can arise without the late components (Fig. 5 A, B) Besides, in a number of cases their thresholds seem to differ lower thresholds which coincide in most cases with subjective auditory thresholds are typical for the early components, the thresholds of the later components being as a rule somewhat higher (Fig. 5 C).

DISCUSSION

The data presented show that in many cases two levels of response "saturation" (or decline) may be traced in the AER amplitude

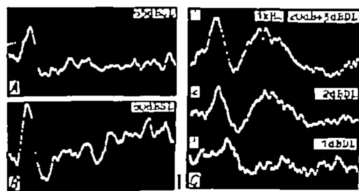


Fig 5 The absence of late AER components at high intensities of sound stimulation (A, B); threshold difference between early and late AER components (C). Amplitude calibration 10 μV time calibration. 100 msec.

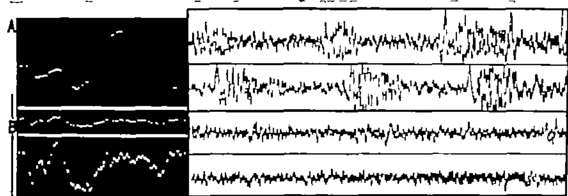


Fig. 6. Left: early and late AER components during synchronized sleep (A) and wakefulness (B); alertness was sustained by counting the number of presented tone bursts. *Right:* EEG recorded during averaging. Two records on *B* are the same AER with different amplification. Amplitude calibration, 10 μV for AER, 50 μV for EEG; time calibration, 100 msec for AER, 1 sec for EEG.

intensity curves: in the range of 40–50 dB SL and 70–100 dB SL. The latter are more consistent and may be better revealed by sounds of higher frequencies; they will be discussed in detail.

The phenomena of AER amplitude "saturation" and decline were revealed with different stimulus presentation intervals, i.e. when tone bursts were presented once every 3.2 or 2.5 sec. This observation contradicts the statement of Picton et al. (1970) that the decline in response amplitude at high intensities is noticeable only when stimuli are presented at intervals of 2.5 sec or less.

According to our results the phenomena of "saturation" and decline in the AER amplitude at high stimulus intensities are statistically significant, although this relation is not so demonstrable and not so easy to reveal as for peak latencies of the early AER components.

The possibility that the amplitude changes of AER at high sound intensities are mediated by the middle ear muscle contractions can be ruled out on the ground of the following considerations: (*a*) the duration of temporal summation in the AER generating mechanisms is probably less than the latency of the middle ear muscle contractions; (*b*) the phenomena of "saturation" and decline in the AER amplitude-intensity curves are more pronounced in cases when auditory stimuli of higher frequencies (e.g. 4 kHz) are used, whereas the contractions of the middle ear muscles affect predominantly the conduction of lower frequencies (see e.g. Wersäll, 1958; Simmons, 1959; Kevanishvili & Gvacharia, 1972).

The character of both AER amplitude and latency changes suggests that "saturation" and decline are postsynaptic phenomena, i.e. they must be attributed to inhibitory processes *within* the AER-generating mechanism itself: to the associative (Davis, 1965) or primary auditory cortex (Vaughan & Ritter, 1970), or to a more complex neuronal network (Davis et al., 1966; Heath & Galbraith, 1966; Khechinashvili et al. 1972).

The importance of central inhibitory processes in the phenomenon of AER "saturation" and decline has been already considered (Picton et al. 1970). However, the possibility was offered that in this event the descending inhibitory elements of the specific auditory system, e.g. the olivo-cochlear bundle, play a major role: the activation of these elements results in a decrease in the output of the specific relays (the cochlea, the cochlear nuclei). According to the presented data such possi-

Acta Otolaryng 76 402–409 1973

LOUDNESS OF BRIEF TONES IN HEARING IMPAIRED EARS

Temporal Integration of Acoustic Energy at Suprathreshold Levels in Patients with Presbyacusis

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(Received March 14, 1973)

Abstract. The temporal integration of acoustic energy has been measured in 24 patients with presbyacusis at 75 and 95 dB SPL, mon- and binaural. The investigation was a loudness estimate of 7 brief tones with a duration of from 5 to 320 ms. Signal presentation was controlled by an on-line computer based on the method of maximum likelihood for estimating psychometric functions. The temporal integration in these patients was not reduced compared with findings in normal-hearing persons. No differences were found monaural versus binaural listening. The findings of this investigation indicate that the temporal integration is not responsible for the reduced discrimination in patients with sensorineural hearing loss.

The hearing threshold is dependent on the stimulus duration in the time domain up to 200 msec. When the pulse duration is decreased, the sound pressure must be increased to reach the threshold. This phenomenon is called temporal integration of acoustic energy and can be quantitatively measured by determination of the threshold shift between tone pulses of different durations.

A threshold shift smaller than found in normals (3 dB per doubling of stimulus duration) is described as reduced temporal integration.

Temporal integration of acoustic energy when measured at threshold, is reduced in patients with sensorineural hearing loss (Hattler & Northern, 1970 Pedersen & Elberling, 1973).

This might be one of the reasons for reduced discrimination in patients with sensorineural hearing loss (Hinchcliffe, 1970), if a difference in temporal integration between normals and patients with sensorineural hearing loss also exists at higher sensation levels.

The purpose of this investigation was to measure the temporal integration at suprathreshold levels in patients with hearing loss of cochlear origin. The results obtained will be compared with findings in normals investigated under similar conditions.

This investigation has been supported by the Danish Government Fund for Scientific and Industrial Research.

MATERIAL

Twenty four persons (13 females, and 11 males) with the diagnosis of presbyacusis participated in the investigation. The age of the test-subjects varied from 60 to 82 years. The test-subjects were selected from the Audiological Clinic, Gentofte Hospital, and most of them had applied for a hearing aid. The diagnosis of presbyacusis was ensured by history otological investigation and audiogram. To ensure an equally composed material, only patients with a symmetric hearing loss of 30–40 dB at 1 000 Hz were investigated.

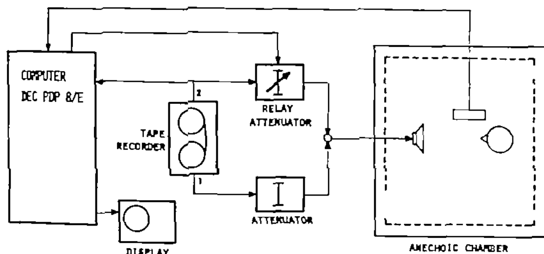


Fig. 1. Block-diagram of the set-up for the loudness balance tests.

METHODS

The investigation was a loudness balance test of tone pulses of varying duration. Tests with mon- and binaural listening were performed at 75 and 95 dB SPL. A block diagram for the set-up is shown in Fig. 1.

Signals

In all investigations, filtered 1 000 Hz tone pulses of the following durations were used: 5, 10, 20, 40, 80, 160 and 320 ms.

Equipment

The pulses were generated by a function generator which started and stopped in zero crossings of the sinus wave. The pulses were filtered through a 1/3 octave filter with a center frequency of 1 000 Hz and were repeatedly recorded on a two-channel tape recorder. Track 1 contained a pulse of a duration T, track 2 contained a pulse of a duration $T/2$, as shown in Fig. 2 (10 ms $\leq T \leq$ 320 ms).

In each test series the tone pulses on track 1 were presented with a fixed intensity. The intensity of the tone pulses on track 2 were changed by a relay-controlled modified HP 350 D attenuator. The smallest step of the attenuator was 1 dB. The dynamic range was from −20 to +80 dB attenuation.

In test series at 75 dB SPL (measured at $T > 320$ ms) an electrostatic loudspeaker (QUAD) was used. In test series at 95 dB SPL (measured at $T > 320$ ms) it was necessary to

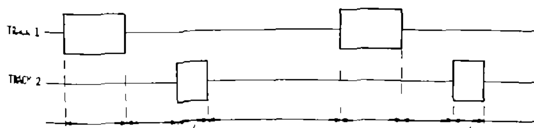


Fig. 2. Sequence of the signals with durations T and $T/2$ (schematic). Actually the signals were filtered before recording.

use an electrodynamic loudspeaker (Lansing S 8) to obtain the high maximum sound pressure level required by the test procedure (115 dB SPL).

The loudness estimations were made in an anechoic chamber (5 × 4 × 3 m³). The test-subject was seated in front of the loudspeaker at a distance of 2 m.

The sound pressure level of the reference tone was controlled by a condenser microphone, replacing the patient s head.

In the monaural investigations a hearing protector (Noisefoe, Mk II) was used on the ear not investigated. This protector will attenuate the sound pressure approximately 40 dB at 1 000 Hz.

In one series, a hearing aid (Oticon 567 PC) was used with a fixed amplification of 20 dB measured in a 2 cc coupler at 1 000 Hz.

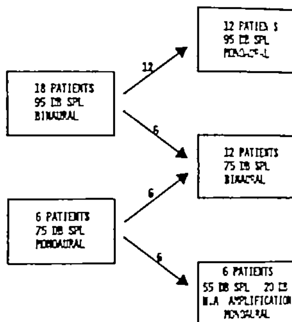


Fig 3 Programme of investigations. The number of patients participating in different test series is shown on the arrows.

Procedure

The test-subject was asked to compare the loudness of 2 tone pulses with a duration of T and $T/2$ (see Fig. 2). His response was given pressing one of three push-buttons:

1) Tone pulse No 1 is the loudest.
2) Tone pulses Nos. 1 and 2 are equally loud.
3) Tone pulse No 2 is the loudest.

The tone pulses were repeated with constant sound pressure levels until the test subject had made a judgement.

After each judgement, the amplitude of the pulse with duration $T/2$ was changed by an on-line computer (PDP 8/E) working on a principle of maximum likelihood for estimating psychometric functions (Hall, 1969; Cocker et al., 1969; Lyregaard & Pedersen, 1971 1973). This method is an adaptive procedure in which the new presentation level is based on the knowledge of previous judgements.

On average, an equal loudness estimation was based on 25 independent judgements per subject.

Two test-subjects were investigated alternatively i.e. after 10 min of loudness judging the test-subject was allowed to rest for 10 min, while the other test-subject was investigated. As mentioned previously 7 different pulse durations were used consequently 6 loudness balance estimates were performed on each subject. Two test-subjects could be investigated during two hours.

Instruction

The test-subjects were instructed before each test. To ensure that the instruction was understood, one or more pilot tests were performed before the results were recorded. One woman was rejected because she was unable to understand the instruction.

Programme of investigation

Fifty four loudness balance test series were performed on the 24 test-subjects. Each series included 6 loudness balance estimates. The programme of investigation is demonstrated in Fig. 3

Eighteen test-subjects were primarily investigated at 95 dB SPL, binaurally Twelve of

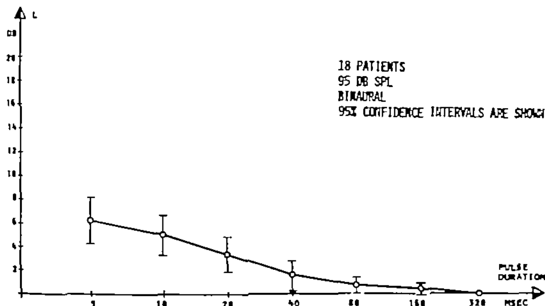


Fig 4

Figs. 4-9 The curves show the increase, *L*, in sound pressure level required to give the same loudness as for a 320 ms tone pulse. *Abscissa:* time in log scale. *ordinate:* sound pressure level in dB.

these 18 test-subjects were furthermore investigated at 95 dB SPL monaurally while 6 were investigated at 75 dB SPL binaurally.

Six test-subjects were primarily investigated at 75 dB SPL monaurally and furthermore investigated at 75 dB SPL binaurally. These 6 test-subjects were also investigated wearing a hearing aid.

RESULTS

It was a characteristic feature in these investigations that a great inter-subject varia-

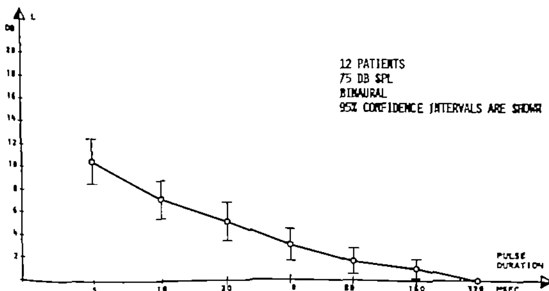


Fig 5

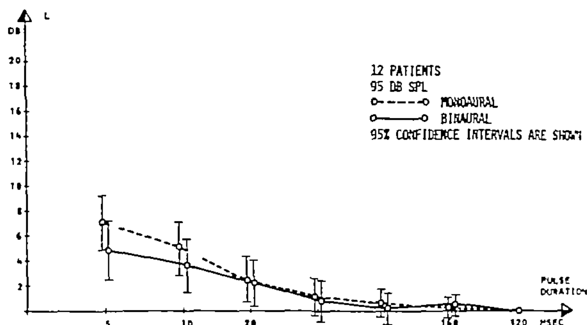


Fig 6

tion existed. The results of 18 test-subjects, investigated at 95 dB SPL binaurally are given in Fig. 4

Twelve test subjects, investigated at 75 dB SPL binaurally showed results as reported in 5 To investigate differences of the tem- integration at mon- and binaural stim- the results from 12 test-subjects in vestigated at 95 dB SPL mon- and binaurally are compared in Fig 6 A similar investigation of mon- and binaural sound application at 75 dB SPL has been made on 6 test-subjects, and the results are given in Fig. 7 To consider the influence of sound intensity at the temporal integration, results from Figs. 4 and 5 may be compared.

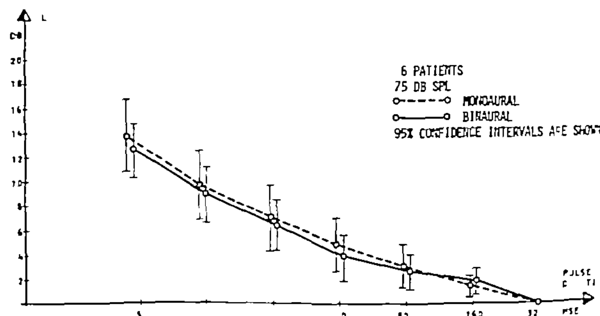


Fig 7

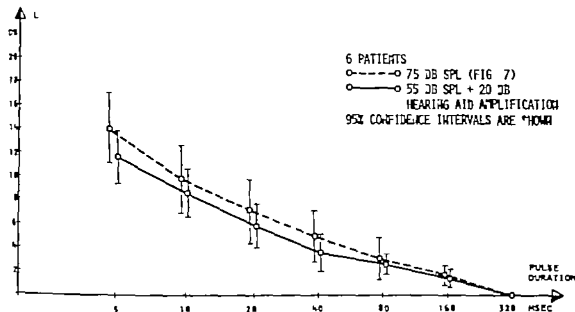


Fig 8

The investigation of 6 test-subjects at 75 dB SPL monaurally and 55 dB SPL + 20 dB hearing aid amplification monaurally showed that the temporal integration was equal under these two conditions (see Fig 8).

DISCUSSION

The reduced ability in patients with a sensorineural hearing loss to discriminate speech can not be explained only by the change in hearing threshold (Pestalozza & Shore, 1955). Factors other than the decrease in hearing threshold must be of importance for discrimination, e.g. a change of the temporal integration and/or a change of the critical band width (Hinchcliffe, 1970).

This investigation has been made to measure the temporal integration at higher sound pressure levels in patients with presbyacusis for later comparison with normals. Several authors have considered the temporal integration in patients with presbyacusis at threshold, and these investigations have shown a reduced temporal integration (Wright, 1968, Hattler & Northern, 1970, Pedersen & Elberling, 1973) but no previous investigations of the temporal integration at higher sensation levels in patients with a sensorineural hearing loss have been reported.

Investigations of loudness summation at various intensities in normals have given the following results. Poulsen (1969) found a marked reduction of the temporal integration at high intensities (70 dB SPL). Lyregaard & Pedersen (1973) investigated the temporal integration on normals at intensities 55, 75 and 95 dB SPL. They used the same method and equipment as reported in this paper and the results are shown in Fig. 9.

Tanemura et al. (1969) found that the temporal integration showed a systematic reduction with increasing sound pressure level. Zwislocki (1969) has mentioned that the temporal integration is reduced at higher sound pressure levels.

From the literature it can be concluded that in normal hearing subjects temporal integration is lower at high intensities than at thresholds.

Our investigation showed that in patients with presbyacusis, the temporal integration was equal to that of normals, when meas

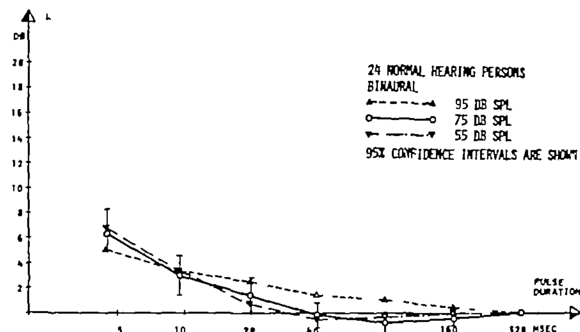


Fig 9

at 95 dB SPL. At 75 dB SPL the temporal integration was found to exceed the values found in normals. Thus, no reduction of the temporal integration in patients with presbyacusis has been found.

Investigations of temporal integration at sensation levels (loudness summation) normals have shown that similar results obtained by mon- and binaural listening (Hellman & Zwislocki, 1963 Rowley & Studebaker 1969). In agreement with this, our investigation showed no significant change at mon versus binaural sound stimulation (Figs. 6 and 7)

By increasing the intensity 20 dB i.e. from 75 to 95 dB a slight decrease in the temporal integration was found (Figs. 4 and 5) This change has not been noticed in normals (Fig. 9) but threshold measurements have indicated a decrease in temporal integration with an increase in SPL in patients with presbyacusis (Pedersen & Elberling 1973)

The use of a hearing aid did not influence the temporal integration in the 6 patients investigated (Fig. 8).

These investigations have shown that the temporal integration in patients with presbyacusis is not reduced compared with findings in normals. This indicates that the temporal integration is not responsible for the reduced discrimination in patients with sensorineural hearing loss.

ZUSAMMENFASSUNG

Ergebnisse von Temporal-Integrationsmessungen an 24 Presbyacusis-Patienten wurden vorgelegt. Die Messungen wurden bei 75 und 95 dB Schalldruckpegel monaural und binaural durchgeführt. Die Untersuchung bestand aus einer Lautstärke Schätzung von 7 Kurztönen mit einer Dauer von 5 bis 320 ms. Die Signaldarbietung wurde von einem on-line computer kontrolliert. Der Computer arbeitete nach dem maximum likelihood-Prinzip zur Schätzung von psychometrischen Funktionen. Die Temporalintegration bei diesen Presbyacusis-Patienten ergab beim Vergleich mit normalen Personen keine Reduktion. Es wurden keine Unterschiede zwischen monauraler und binauraler Schallstimulierung gefunden. Die Resultate dieser Untersuchung zeigen, dass die veränderte Temporalintegration nicht für die Diskriminationsschwierigkeiten bei Presbyacusis-Patienten verantwortlich sein kann.

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Acta Otolaryng 76 410–418 1973

EFFECT OF POTASSIUM DEFICIENCY ON COCHLEAR POTENTIALS AND CATION CONTENTS OF THE ENDOLYMPH

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(Received April 11 1973)

Abstract The scala vestibuli and/or scala tympani in anesthetized guinea pigs was perfused with potassium-free solution. Cochlear microphonics (CM), summating potential (SP), action potential (AP) and endocochlear potential (EP) were recorded before during and after the perfusion. The sodium and potassium contents in the endolymph were also determined. Perfusion of both scala vestibuli and tympani with control solution did not produce appreciable changes in electrical responses or sodium and potassium content in the endolymph. Perfusion of the perilymphatic space with potassium-free solution caused significant suppression of EP and AP The CM was also reduced and accompanied by increase of the positive SP These changes were reversible The measurement of potassium and sodium content in the endolymph indicated that potassium deficiency in the perilymph resulted in significant increase of sodium and decrease of potassium content. The role of potassium ions in the perilymph is discussed.

Since Smith et al. (1954) demonstrated the unique high content of potassium in the endolymph a great deal of attention has been directed toward the role of potassium ions in the endolymph in maintaining the cochlear potentials. Konishi et al. (1966) reported that potassium-rich endolymph is essential for maintaining the high sensitivity of the hair cells of the organ of Corti.

The effect of an increase in potassium content in the perilymph on the cochlear potentials has also been examined by several investigators (Tasaki & Fernández, 1952 Kuijpers, 1969) their results indicate that increased potassium concentration in the scala tympani causes suppression of both cochlear microphonic (CM) and the whole-nerve action potential (AP) and a temporary increase of the endocochlear potential (EP) However the effect of potassium deficiency in the perilymph on the cochlear potentials has not yet been fully investigated. High Na K -activated enzyme activity was found in the stria vascularis (Kuijpers, 1969· Nakai & Hilding 1966) and in view of this the stria vascularis appears to be the site of the cation pump which maintains the ionic difference between the endolymph and the perilymph (Iinuma, 1967 Kuijpers, 1969 Bosher & Warren, 1968). As the Na K -activated enzyme system requires for activation potassium and sodium in addition to magnesium the concentration of potassium in the perilymph would be critical for maintaining the activity of the stria vascularis, and potassium deficiency would lead to the deterioration of cochlear function

The present paper deals with changes of cochlear potentials and ionic composition of the endolymph when the perilymph is replaced with a potassium-deficient solution and provides the evidence that the normal function of the cochlea is reversibly suppressed

The present investigation was supported by the National Institute of Neurological Disease and Stroke NS-05016.

[1] Also from Department of Physiology and Communication Sciences Laboratory

after elimination of potassium ions from the perilymph. The abstract of this paper was presented at the 83rd meeting of the Acoustical Society of America in 1972.

METHODS

Healthy guinea pigs anesthetized with pentobarbital sodium were used throughout the experiments. The cochlear potentials were recorded from the basal turn with differential electrodes (Tasaki et al. 1952). The technique for recording the cochlear potentials including CM, summating potential (SP), AP and EP has been fully described in our other papers (Konishi et al. 1961). In some cases, CM and EP were recorded from the basal and third turns simultaneously. In the third turn it is difficult to prevent leakage of the perfusate from the holes into which the differential electrodes are inserted, thus CM from the third turn, when necessary, was recorded from the scala media. The tone bursts used as acoustic stimuli were delivered in a closed system.

The techniques of sample collection and analysis of endolymph used in this experiment were essentially the same as those described elsewhere (Mendelsohn & Konishi, 1969). If the sample was collected smoothly, EP did not show significant changes. When EP showed a sudden drop or decreased continuously by more than 10 mV, the sample was discarded.

The perfusion technique used in this experiment has also been described in our previous paper (Konishi & Kelsey, 1968 *a*). The rate of the perfusion was approximately 2 μl/min and the period of perfusion was 20 min. The artificial perilymph had the following composition (mM): NaCl 137, KCl 5, $CaCl_2$ 2, NaH_2PO_4 1, $MgCl_2$ 1, $NaHCO_3$ 12, glucose 11. The pH of this solution ranged from 7.2 to 7.4. Potassium-free solution was made by eliminating KCl in the above solution and the isotonicity of this solution was maintained by adding the appropriate amount of glucose. The isotonicity of these solutions was determined by freezing-point measurement. The perfusate was kept at room temperature (20 C).

Procedure

The anesthetized animal was immobilized with an intravenous injection of gallamine triethiodide. The experiments were carried out under artificial respiration. In the preparations which were designed to examine the temporal changes of the cochlear potentials the first perfusion, except for a few cases, was carried out with the artificial perilymph for a period of 20 min. About 10 to 30 min later *potassium-free solution* was introduced into the perilymphatic space and again about 10 min later washing with the artificial perilymph was carried out. The period of the second and third perfusions was the same as the first. The electrical responses were recorded periodically before, during, and after the perfusion.

In the preparations which were used for determination of potassium and sodium content in the endolymph, the sample of the endolymph was taken immediately after the end of the perfusion with either the control or the potassium-free solution. The CM and AP were monitored during the perfusion but EP was not measured continuously in these cases.

RESULTS

Alteration of cochlear potentials

Perfusion with artificial perilymph. It has been shown in our previous papers (Konishi & Kelsey, 1968 *a*; Konishi, 1972) that the cochlear potentials did not change appreciably during perfusion when artificial perilymph was introduced into the perilymphatic space at the rate described in *Methods*. When the sound-evoked responses were recorded from the basal turn, the input–output curve of CM and AP at 6 kHz showed essentially the same configuration before and after the

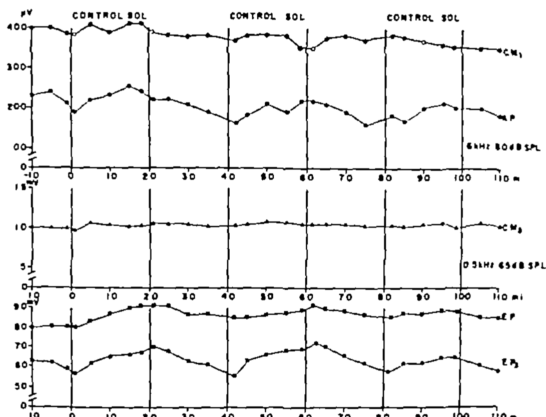


Fig. 1. One example showing changes in cochlear potentials during and after three consecutive perfusions of scalae vestibuli and tympani with artificial perilymph. CM_1, CM recorded with differential electrodes placed in the basal turn; CM_3, CM recorded with a microelectrode inserted into the scala media of the third turn. EP and EP are recorded from the basal and third turn respectively

) lso the isopotential curve of CM at the level of 400 μV was hardly affected by the perfusion and its difference fell within ±1 dB over the range of 0.5 to 10 kHz.

The slight changes in the cochlear potentials seen in the first perfusion could be reproduced by subsequent perfusions. Fig. 1 shows one example of changes in cochlear responses during and after three consecutive perfusions of scalae tympani and vestibuli. The CM recorded in the basal turn and third turn CM_1 and CM_3 respectively were little affected by any of the three perfusions. The time course of AP was similar in each perfusion. Also EP in the basal and third turns demonstrated a slight increase during the perfusion and a gradual return after the perfusion even though the magnitude of changes was greater in the third than in the basal turn.

It should be noted that there is little cumulative effect on the coclear potential of the three consecutive perfusions. Thus, it can be safely assumed that the effect of the potassium-free solution on the cochlear potential could be estimated by comparing the changes

Table I

		Endolymph			
		K mEq/l	Na mEq/l	EP (mV)	N
Normal	$\bar{X}$	161.65	1.04	83.4	10
	σ	3.82	0.51	6	
Control sol.	$\bar{X}$	166._5	1.30	8_.4	10
	σ	8.1	0.57	4.1	
K-free sol	$\bar{X}$	151.65	3.55	43.8	10
	σ	14.16	_.6_	1.8	

Significant t: $p < 0.05$

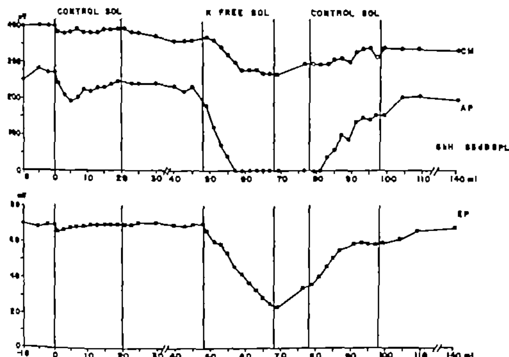


Fig. 2. Changes in CM, AP and EP produced by perfusion of the scalae vestibuli and tympani with potassium-free solution. All responses are recorded from the basal turn. Note the rapid recovery of the responses during washing with artificial perilymph.

in the first perfusion with the normal perilymph with those observed in the second perfusion with the potassium-free solution.

Perfusion with potassium-free solution. The cochlear potentials, EP and the sound-evoked responses, showed marked changes during and after the introduction of potassium-free solution into the perilymphatic space. A typical example is shown in Fig. 2. EP began to drop progressively within 5 minutes after the perfusion and declined progressively as the perfusion continued. In most cases EP was suppressed to about 50% of the pre-perfusion magnitude at the end of the perfusion. Even though the rate of the decline in EP varied among the preparations, the suppression of EP was consistently observed during the perfusion. Recovery of EP after the perfusion was usually rapid and in most cases partial recovery was recorded even without washing with the artificial perilymph. With washing EP recovered fully to the pre-perfusion level.

CM was also suppressed by perfusion with potassium free solution. Strikingly the decline of CM was usually slower than that of EP. CM remained unchanged for 5 to 10 min after the perfusion. There was no consistent parallel relationship of decline between CM and EP. As the perfusion continued, CM began to decline and at the end of the perfusion the loss of CM was about 20%. The input-output curve of CM taken before and immediately after the perfusion showed lateral shift of the linear portion of the curve by about 3 dB and suppression of its maximum peak (Fig. 3). The isopotential curve for CM at the 400 μV level showed on the average a 3 dB loss in the frequency range from 0.5 to 10 kHz. Recovery of CM was observed after the perfusion but only a few cases showed complete recovery of CM after washing.

The changes in SP were characteristic. In all cases in which EP declined progressively during the perfusion the positive SP was prominent. The changes in SP were similar to

those observed during asphyxia (Konishi et al., 1961) or perfusion with metabolic inhibitors (Konishi & Kelsey 1968 *b*) SP had a tendency to increase its negativity during the recovery period of CM or EP

AP was depressed much faster than were other electrical responses (Fig. 2). AP showed a suppression greater than 50% after 10 min of perfusion. At the end of the perfusion, in the majority of cases, AP could be observed only at high intensities of stimuli as shown in Fig. 3 AP recovered rapidly after the end of the perfusion and occasionally super-normality of AP was observed after washing.

The changes in EP CM and SP in the third turn showed a time course similar to those recorded in the basal turn. The input-output course of CM response to 0.5 kHz demonstrated changes similar to those recorded in the basal turn.

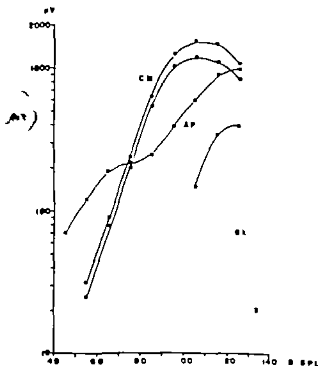


Fig 3 Comparison of input-output curves of CM (circles) and AP (rectangles) before and after the perfusion with potassium-free solution Filled symbols, pre-perfusion open symbols, post-perfusion Responses are recorded from the basal turn Acoustic stimulus, 6 kHz tone burst, rising and falling time 0.5 msec

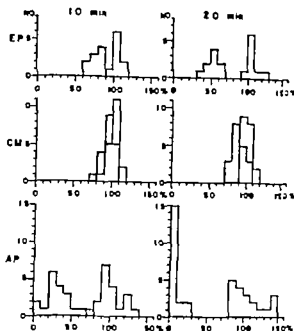


Fig 4 Frequency diagrams showing the changes in EP CM and AP 10 min (left side) and 20 min (right) after initiation of perfusion with artificial perilymph (blank column) and potassium-free solution (shaded column). The ordinate is number of cases and the abscissa is changes in responses expressed in percent. The magnitudes of responses before perfusion are taken as 100%

The frequency diagrams, as shown in Fig. 4 demonstrate the changes, in CM and AP in response to 6 kHz tone bursts at 80 dB SPL, and in EP recorded in the basal turn at 10 and 20 min after the perfusion with the artificial perilymph (blank columns) and the potassium free solutions (shaded columns). The changes in responses are expressed in percentage The responses at the pre-perfusion time are taken as 100% It is noted that EP and AP were suppressed progressively during the perfusion with potassium-free solution but the suppression of CM was less pronounced.

It has been reported by several investigators that Reissner's membrane plays a major role for active transport (Rauch et al., 1963). Therefore perfusion with potassium-free solution was carried out in the scala vestibuli or tympani separately The suppression of EP

or CM was less in both cases than with perfusion of the entire perilymphatic space. However our results did not show more rapid decline or more marked loss of these responses in perfusion of the scala vestibuli than in that of the scala tympani. In both of the separate perfusions, EP decreased its magnitude to 70 to 80% and loss of CM was approximately 5%. AP showed a more rapid decline during the first 10 minute period of perfusion of the scala tympani than it did in that of scala vestibuli but loss of AP was about 30% at the end of the perfusion.

In order to answer a question as to whether the cochlear aqueduct may reduce the effectiveness of the perfusion of the perilymphatic space, surgical obstruction of the cochlear aqueduct was performed. Our results indicate that the effect of the perfusion on the cochlear potentials was not enhanced by the obstruction of the cochlear aqueduct, when the perfusion was carried out as described in *Methods*.

Alteration of endolymph composition

In order to evaluate the effect of potassium deficiency in the perilymph on the concentration of sodium and potassium of the endolymph, a comparison was made in three groups; one without perfusion, the second with perfusion with the artificial perilymph and the third with perfusion with the potassium-free solution. A summary of the results with respect to endolymph is shown in Table I. The mean and standard deviations were obtained from 10 experiments in each group. All data included in this table were obtained without a loss of EP greater than 10 mV during the collection.

In normal preparations, the mean value for sodium concentration was slightly lower and that of potassium higher than that reported by us previously (Mendelsohn & Konishi, 1969). These values were found to be comparable to those obtained in adult rats by Bosher & Warren (1968). The perfusion with the artificial perilymph did not alter the sodium or potassium content of the endolymph. Statistical treatment with the *t*-test revealed that there were no significant differences between normal and control groups at the 5% level.

In the third group, using perfusion with potassium-free solution, the same procedures were employed as in the perfusion with normal solution. However the results indicated a decrease of potassium and an increase of sodium content after removal of potassium ions from the perilymph. These differences were found to be significant at the 5% level.

The magnitudes of EP obtained during the collection of the endolymph also indicate that perfusion with artificial perilymph results in no significant alterations of EP from those obtained from non-perfused cochlea. However perfusion with potassium-free solution decreased EP significantly according to the *t*-test.

DISCUSSION

The present results suggest that removal of potassium ions from the perilymph results in reversible suppression of the cochlear potential and that the optimal concentration of potassium ions in the perilymph is essential for the generation of the cochlear potentials. This raises a question as to the effect of potassium deficiency on transducer action of the cochlea. While the effect of increase of potassium concentration in the perilymph can be attributed to the depolarization of the hair cells and the primary neurons, the effects of removal of potassium ions are less easily explained. Several possible mechanisms should be considered. (1) suppression of active transport, (2) enhancement of the endolymph-negative diffusion potential, and (3) depolarizing action.

K^{42} is rapidly secreted from the blood into the endolymph, while it enters the perilymph more slowly (Choo & Tabowitz, 1965) and labelled potassium is transferred quickly from the scala vestibuli to scala media (Rauch et al. 1963). These findings strongly suggest that the high potassium content of the endolymph

is maintained by the active ion transport and the EP is essentially the secretion potential generated by an electrogenic cation pump. This concept has been supported by findings that ouabain, a specific inhibitor of Na K activated enzyme suppressed EP and causes rise of sodium and fall of potassium in the endolymph (Konishi & Mendelsohn 1970; Kuijpers, 1969). The Na K-activated enzyme requires for activation potassium and sodium in addition to magnesium and this is nearly maximally activated at 5 mM of potassium which is the normal concentration in the perilymph (Kuijpers, 1969). Thus it is likely that the replacement of the perilymph with potassium-free solution results in suppression of ATPase activation which reflects the drop of EP and fall of potassium in the endolymph. Kuijpers (1969) and Iinuma (1967) reported the strong activity of ATPase in the stria vascularis and Nakai & Hilding (1966) found that ATPase activity is located on the cell membrane of the marginal cells of the stria vascularis and in the basement membrane of the capillaries.

Our results seem to indicate that the stria vascularis is primarily involved by removal of potassium in the perilymph because the perfusion of the scala vestibuli with potassium-free solution did not suppress EP more quickly or markedly than did that of scala tympani. Although the role of Reissner's membrane cannot be ruled out in terms of ion active transport (Hinojosa, 1972), it seems likely that the transport of potassium from the scala vestibuli to the endolymph, reported by Rauch et al. (1966) did not occur across Reissner's membrane but through the stria vascularis.

The second explanation would be that the decrease of EP by removal of potassium may reflect the combined effect of suppression of the positive secretory potential and enhancement of the endolymph-negative diffusion potential. As it is generally assumed that the organ of Corti is filled with perilymph or perilymph-like fluid, Kuijpers (1969) suggested that EP is normally balanced by the positive secretory potential and the negative diffusion potential produced by the potassium concentration gradient between endolymph and perilymph or blood. He found that the negative potential which appeared in the scala media during the early stage of asphyxia can be eliminated by increasing the potassium content in the perilymph. As the positive secretory potential is sensitive to the lack of oxygen supply and the potassium concentration in the endolymph remained unchanged during the early stages of asphyxia, the negative potential in the asphyxiated scala media may be attributed to the potassium diffusion potential across the membrane separating endolymph from the perilymph or blood. However Konishi et al. (1967) found that the hair cells of the organ of Corti can respond to externally applied d.c. polarization or static displacement of the basilar membrane. This finding suggests the possibility that the application of potassium-rich solution to the perilymphatic space in the asphyxiated cochlea alters the responsiveness of the hair cells. This possibility is in line with our previous finding (Konishi et al. 1967) that the magnitude of the negative EP in the asphyxiated cochlea is smaller in kanamycin-treated animals than in normal, although potassium content in the endolymph in kanamycin intoxicated animals was found to be in the normal range (Konishi, unpublished). From these previous observations, it is still not clear that removal of potassium from perilymph enhances the potassium diffusion potential across the cochlear partition. Further experimental data for these phenomena must be acquired.

It is expected from the Goldman equation that the removal of external potassium ions would produce hyperpolarization of the membrane if little change in ionic permeability occurs. Recent studies on taenia coli of guinea pigs (Tomita & Yamamoto, 1971) demonstrate the depolarization in removal of external potassium. One possible explanation is that when the external potassium concen-

tration is reduced, the potassium conductance of the membrane is so reduced that the effect of the increase in potassium equilibrium potential is counteracted (Hall et al., 1963). It is likely that removal of potassium from the perilymph affects the auditory nerve or end legs but it is uncertain whether removal of external potassium ions affects the transducer mechanism in the same way that it does the impulse-generating membrane.

Deficiency of potassium ions in the perilymph might result in an increase in permeability of the cochlear partition and consequently potassium and sodium ions diffuse passively in their concentration gradient. As a result the potential loss in the scala media does not reflect the decrease in EMF of the stria vascularis but merely indicates the decrease of IR drop across the cochlear partition. This possibility still remains to be answered until the ionic permeability of the cochlear partition is quantitatively examined in normal and pathological conditions.

The present study clearly demonstrates the importance of potassium ions in the perilymph for maintaining the normal function of the cochlea and suggests the possibility of occurrence of sensori-neural hearing loss by potassium deficiency in the perilymph.

ZUSAMMENFASSUNG

Die Scalae vestibuli bzw. tympani anästhesierter Meerschweinchen wurden mit kaliumfreier Lösung durchspült. Die Cochlearmikrophonie (CM), das Summationspotential (SP), das Aktionspotential (AP) und das Endocochlearpotential (EP) wurden vor, während und nach der Durchspülung aufgezeichnet, und der Natrium- und Kaliumgehalt wurde bestimmt. Die Durchspülung sowohl der Scala vestibuli als auch der Scala tympani mit einer Kontrollösung rief keine merkbaren Veränderungen im elektrischen Verhalten oder im Natrium- oder Kaliumgehalt der Endolymphe hervor. Durchspülung des perilymphatischen Raums mit kaliumfreier Lösung verursachte eine weitgehende Unterdrückung des EP's und des AP's. Die CM wurde ebenfalls herabgesetzt, bei gleichzeitiger Erhöhung des positiven SP's. Diese Veränderungen waren auch umkehrbar. Eine Messung des Kalium- und Natriumgehalts der Endolymphe ergab, dass ein Kaliummangel in der Perilymphe eine beträchtliche Erhöhung des Natriumgehalts und eine erhebliche Verminderung des Kaliumgehalts hervorrief. Behandelt wurde auch die Rolle der Kaliumionen in der Perilymphe.

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Acta Otolaryng 76 419–425 1973

THE THRESHOLD OF OCTAVE MASKING (TOM) TEST

Further Observations

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(Received March 4 1973)

Abstract. A threshold of octave masking test (TOM) has been suggested as a substitute for measuring aural harmonic thresholds. The test involves measuring masking at one octave above the masker (*M*). This study was conducted to investigate further the value of the TOM test in differential diagnosis. A group of normal and sensori-neural impaired listeners were administered the TOM at *M* conditions 500, 1 000, and 2 000 Hz. The results showed significant (0 05) differences between the normal and abnormal hearing groups. Excellent test-retest reliability at all *M* conditions was reported. When the TOM data for the two groups were compared with the aural harmonic thresholds reported by previous researchers, close agreement was found to exist. Thus, aural harmonic levels and TOM values appear to provide the same information. The TOM is easy to administer does not require sophisticated equipment, and data can be obtained in a very short period of time. Though continued research is necessary this test appears to have potential as a special test in audiometric diagnosis.

The measurement of aural harmonic thresholds has demonstrated excellent potential as a diagnostic tool in audiology Such measurements provide information as to the functioning of ears about midway between absolute and discomfort thresholds. A number of investigators have demonstrated that subjects with cochlear involvement yield lower harmonic thresholds than normal hearing persons (Opheim & Flottorp, 1955 Lawrence & Yantis, 1956 Lawrence, 1958, Yantis et al., 1966) Two studies have reported that aural harmonic threshold measurements can be used as a substitute for recruitment tests (Sokolowski, 1951 Opheim & Flottorp, 1955). Aural overload testing has also been used to predict susceptibility to acoustic trauma (Lawrence & Blanchard, 1954) to estimate the amount of cochlear reserve in otosclerosis (Yantis & Magielski, 1958) and to determine whether amplitude distortion is a contributing factor to discrimination breakdown at intensity levels above the PB max (Yantis et al. 1966).

Though harmonic threshold measurements appear to have value, few audiologists include this test among their clinical battery The probable reason for this neglect seems to be the difficulty involved with obtaining accurate harmonic threshold determinations. The psychophysical technique most commonly used to measure harmonic thresholds is the exploring tone method. Very simply this technique involves introducing a fundamental tone to the ear at an intensity sufficient to produce a second aural harmonic. To assist in the detection of this harmonic, an exploring tone, varying in frequency from the aural harmonic by only a few cycles, is introduced to the ear This exploring tone then bea

The investigation was supported, in part, by the Central Michigan University Faculty Research and Creative Endeavors Committee

This article is based on the senior author's Master's thesis under the direction of Dr Fred H Bess.

aural harmonic and is heard in the back ground of the fundamental. The use of this method for detecting harmonics however has been subjected to considerable criticism. For example Békésy (1957) concluded that measurements of the threshold and growth rate of aural harmonics are contaminated by interactions of frequency discrimination. Egan & Klumpp (1951) demonstrated an over estimation bias inherent in the exploring tone procedure. Finally there are practical difficulties involved with making such measurements. Harmonic threshold determinations have been reported to take as long as 30–45 min depending on the experience motivation, and intelligence of the listener (Clack & Bess, 1969)

Recently Clack & Bess (1969) proposed a simpler alternative tone-on-tone masking technique which was found to produce results essentially equivalent to the exploring tone method. The rationale for this technique was based on a series of earlier experiments by Clack (1967 1968 *a* 1968 *b*). He revealed that harmonic distortion begins and grows at a rate equal-to or less-than masked thresholds ow 70 dB SL) Based on these data, Clack Bess (1969) reasoned that harmonic thresholds and masking thresholds may be equiv alent if the masking was measured in the frequency vicinity of the aural harmonic. Accordingly this test involved measuring the monaural masking at one octave above the fundamental masker (Mf). Using 1 000 Hz as the Mf_n Clack & Bess (1969) found that the intensity level first needed to cause a threshold shift in the maskee (Mf 2 000 Hz) was greater for normals than it was for a sensori-neural impaired group Thus, less Mf intensity in sensation level (SL) was needed with abnormal hearing listeners to produce a small threshold shift in the Mf_r. The intensity level first needed to cause a shift in the Mf was referred to as the threshold of octave masking (TOM).

The purpose of this research was to further evaluate the usefulness of the TOM for differential diagnosis. The present study endeavored to eliminate certain limitations found in the initial investigation by incorporating a larger N more Mf and Mf frequencies, and choosing subjects with clearly defined lesions of the cochlea

METHODS

Subjects

The subjects in the present investigation consisted of a normal and a sensori-neural impaired population. Ten subjects (4 males and 6 females) ranging in age from 20–34 years (mean 24 years) made up the normal hearing group. Each of these subjects passed an audiometric screening at 10 dB ISO for octaves ranging from 125–8 000 Hz. Twelve persons (8 males and 4 females) ranging in age from 11–83 with a mean age of 59 years, served as paid listeners in the group with sensori-neural involvement. All subjects in the sensori-neural group were selected from patient files at the Central Michigan University Hearing Clinic. The following criteria were used in the selection of these subjects:

1 Pure tone thresholds of at least 25 dB ISO at frequencies 500–4 000 Hz
2. A positive indication of cochlear involvement as evidenced by three or more of the following:
    (*a*) Complete or partial recruitment on the ABLB test (Fowler 1928)
    (*b*) A score of 60–100% at 2 000 Hz and 4 000 Hz on the Short Increment Sensitivity Index (Jerger et al. 1959)
    (*c*) No evidence of tone decay at 2 000 Hz and 4 000 Hz (Carhart 1959)
    (*d*) Type II Békésy (Jerger 1960)
    (*e*) A negative Modified SISI at 2 000 Hz and 4 000 Hz (Thompson, 1963).

From the 12 persons selected for this group, a total of 20 ears satisfied the above criteria and were used in this study A summary of the diagnostic tests used in the selection of

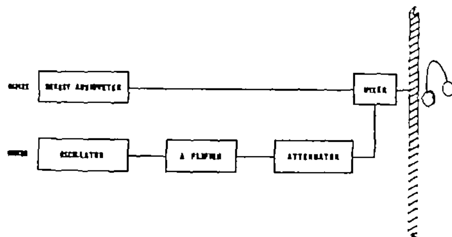


Fig. 1. Schematic of apparatus used to measure the threshold of octave masking.

the subjects with sensori-neural involvement is presented in Table I.

Apparatus

A schematic of the equipment used in the TOM test is seen in Fig. 1. The *M* tone was generated by a Grason-Stadler (Model E800) Békésy audiometer fed through a mixer and impedance matching network, through the wall of an IAC booth and directly to a calibrated TDH-39 earphone mounted in a MX 41/AR cushion. The *M* tone was generated by a General Radio (Model 1309-A) oscillator, followed by an amplifier (Dynaco SCA 35) and a Davon attenuator (Model T-693-R). The signal was then fed to the mixing network, through the wall of the IAC booth to the same TDH-39 earphone. A Grason-Stadler (Model 162) speech audiometer was used to maintain voice communication between the subject and the examiner.

Pre-experimental calibration showed that the acoustic harmonic output at the earphone was more than 60 dB below each of the *M*'s. The linearity of both the Békésy audiometer and the Davon attenuator was checked and found to be linear. The frequency of the *M* and *M* tone was adjusted prior to and during each run using an Eldorado (Model 1607) frequency counter. The ambient noise levels in the IAC booth were recorded at octaves ranging from 62.5 to 8 000 Hz and found to conform to ANSI (1960) standards.

Procedure

Prior to the test sequence each subject was instructed in the usual manner for Békésy audiometry. In addition, each subject was directed to disregard the steady tone completely and listen only for the rhythm of the pulsed tone.

Table I. *Diagnostic summary of sensori-neural impaired listeners*

Subject	Age	Sex	Speech discrimination scores: Right	Left	Positive audiometric finding
E. A.	72	♂		86	*a, b, d*
F. M.	83		90	96	*a, b, d*
E. M.	55	♂	92	84	*a, d*
F. V.	79		63		*a, b, d*
E. B.	54	♂	88	80	*a, c, d*
S. O.	12	♀	84	96	*a, c, d*
C. R.	50	♂	92	60	*a, c, d*
W. D.	52	♂	96	90	*a, b, d*
L. W.	56	♂	68	80	*a, b, d*
J. S.	77	♂	76		*a, b, d*
A. J.	59	♀	84	68	*d, c*
W. B.	62	♂	80		*a, c, d*

a – Short Increment Sensitivity Index.
b – Modified Short Increment Sensitivity Index.
c – Békésy Audiometry.
d – Tone Decay Test.
e – Alternate Binaural Loudness Balance.

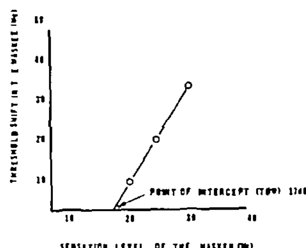


Fig Example of procedure used to establish the threshold of octave masking. The point of interception is considered the level at which masking first begins (TOM).

The *M* thresholds were then measured utilizing the modified Hughson-Westlake technique as described by Carhart & Jerger (1959) After the *M* thresholds were obtained the subject was instructed to begin to trace his threshold for the *M* tone using a Békésy automatically recording audiometer

Each subject was allowed to practice for a period of time to make sure he under [illegible]d the listening task After the threshold [illegible] the *M* had been established (about 10–15 sweeps of the attenuator) the examiner introduced the *M* The *M* was subsequently increased to a point where a shift of at least 10 dB (SL) was noted in the threshold of the M_r. Once this shift was established the intensity of the *M* was increased in two 5 dB increments. This entire procedure was then duplicated for each test run for the *M* frequencies of 500 Hz, 1 000 Hz, and 2 000 Hz with the *M* frequencies one octave above each M_r. Following a short rest period each subject received a retest on all listening conditions.

RESULTS

Thresholds were determined by drawing a line through the midpoint of the last 10 pen excursions. The resulting threshold shifts for a given *M* condition were plotted on a graph as shown in Fig. 2. Using an assumption of masking linearity a straight line was drawn between the two extreme threshold values and then extrapolated to intercept with the abscissa. The value at the point of interception became the TOM

Table II *Mean threshold of octave masking (TOM) values and standard deviations for the normal and sensori-neural impaired listeners*

Group		Frequency of the masker		
		500	1 000	[illegible]000
Normal-hearing	Mean S.D	44 (6.8)	53 (6.0)	56 (7.8)
Sensori-neural	Mean S.D	16 (16.7)	28 (21.4)	16 (1[illegible].7)

The obtained mean TOMs and standard deviations for the normal and sensori-neural impaired populations are summarized in Table II Predictably significant (0.05) differences were found between the two groups at all *M* conditions. Standard deviations obtained at all three *M* s indicated considerable variability in both groups, however the dispersion was much greater in the subjects with cochlear involvement.

An examination of some individual data from the two groups illustrates the variability seen in these subjects. Figures 3 and 4 represent the TOM values for three randomly selected subjects within each group at all *M* conditions. In the normal hearing subjects (Fig. 3) the TOM values seem to vary by as much as 15 dB In the abnormal hearing group (Fig. 4) the TOM values at 500 Hz range from 10–26 dB while at 1 000 Hz and 2 000 Hz the threshold v[illegible] qui[illegible] as large It is also [illegible] that a few of the normal [illegible] hibited TOM values of [illegible] sequently in some [illegible] for subjects with [illegible] proximated the [illegible]

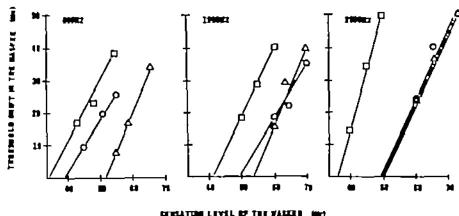


Fig. 3 Threshold of octave masking values for three randomly selected normal hearing subjects at all *M* conditions.

hearing subjects. This finding, however was found to occur in only a few instances.

Test-retest reliability in both groups was examined using the Pearson Product Moment Correlation (Garrett, 1958) The results of this analysis as well as levels of expectation necessary for obtaining significance (0.05) are shown in Table III. Significantly high correlations were found in both groups, indicating excellent test-retest reliability

The TOM values obtained in this investigation at 1 000 Hz are in close agreement with the data reported by Clack & Bess (1969). The mean TOM values for their normal and abnormal hearing groups were 53 and 26 dB while the mean TOMs in the present study were 53 and 27.5 dB respectively Although the Clack & Bess (1969) data were not subjected to a formal test of reliability they also reported extremely close test-retest scores for both groups. It needs to be pointed out, however that the range of TOMs obtained in the Clack & Bess (1969) study for the normals as well as the abnormals did not show the variability obtained in the present study In the normal-hearing listeners they reported a range of 8 dB compared to 28 dB obtained for this study This may be the result of the smaller samples used in the Clack & Bess (1969) investigation. Comparable differences in variability were also seen among the cochlear impaired groups.

DISCUSSION

The results of this investigation have shown that the TOM test will differentiate subjects with cochlear involvement from normal hearing subjects at all three *M* frequencies. In addition, the test has demonstrated excellent test retest reliability

The tone-on-tone masking technique seems to have certain clinical advantages over other special tests currently used in hearing clinics. For example, the TOM can be administered in cases with both unilateral and bilateral hearing losses. Further the test does not require complicated or expensive instrumentation and has been found to be quick and easy to administer Finally the listening task is not difficult. Any person capable of performing routine sweep-frequency Békésy-type audiometry can be tested without practice.

In the initial TOM investigation Clack Bess (1969) hypothesized that octave masking and harmonic pear at the same *M* ... amine this hypothesis it pare the aural

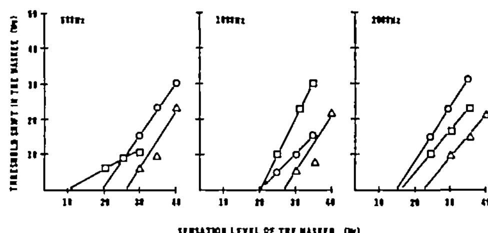


Fig 4 Threshold of octave masking values for three randomly selected sensori-neural impaired subjects at all *M* conditions.

tained by previous investigators to the TOM values obtained in the present study Table IV presents such a comparison using the harmonic measurements reported by three researchers. It is apparent from this table that the harmonic thresholds and TOM values are very similar in the normal group for all three frequencies. Moreover the TOM values are fairly compatible with the harmonic thresholds seen in the sensori-neural impaired listeners although there is more variability Thus, these data lend further support to the contention that TOM values and aural harmonic levels, as measured by the exploring tone method yield essentially the same data.

Before this test can be used in a clinical setting with any degree of confidence additional study is needed using larger samples with a variety of hearing disorders. In particular data are needed in subjects with purely conductive and VIII nerve lesions. The effects of hearing loss at the *M* and *M* also warrant investigation.

Table III *Correlations obtained between test and retest conditions for the normal and sensori-neural impaired listeners*[a]

	Frequency in Hertz		
Group	500	1 000	000
Normal-hearing	.93 (.44)	.85 (.44)	.96 (.44)
Sensori-neural	.82 (.55)	.93 (.49)	.93 (.45)

[a] The lowest correlation coefficient which will indicate significance at the 0.05 level of confidence for the N used in the analysis appears in ( ).

Finally there needs to be additional research conducted on the number of masked data points required to obtain an accurate TOM value A basic assumption of the TOM is that masking grows linearly once an initial shift in the *M* occurs.

If the intensity levels of the *M* are not sufficient to produce a linear function, then the extrapolation procedure will cause an underestimation of the TOM value Hence it seems important to investigate how greater *M* intensity levels can affect the TOM.

In conclusion, this study further examined the usefulness of the TOM test in differential diagnosis. The results indicated that the test was capable of differentiating between normal hearing persons and subjects with cochlear involvement. The test was found to be quick and easy to administer The results of the study were in agreement with the initial investigation by Clack & Bess (1969) and also with the harmonic thresholds reported by previous investigators (Opheim & Flottorp.

Table IV *Mean aural harmonic thresholds reported by three previous investigators and mean threshold of octave masking values obtained in the present study*

		500 Hz	1 000 Hz	2 000 Hz
Harmonic Thresholds				
Lawrence & Yantis (1956)	Normal	—	52 dB	57 dB
	Sensori-neural	—	17 dB	23 dB
Yantis, Millin & Shapiro (1966)	Normal	47 dB	53 dB	50 dB
	Sensori-neural	28 dB	30 dB	28 dB
Opheim & Flottorp (1955)	Normal	42 dB	—	—
	Sensori-neural	10 dB	—	—
TOM Values				
Grimm & Bess (1973)	Normal	44 dB	53 dB	56 dB
	Sensori-neural	16 dB	28 dB	16 dB

1955 Lawrence & Yantis, 1956 Yantis et al., 1966)

ZUSAMMENFASSUNG

Eine Schwelle des oktavmaskierten Versuchs (TOM = threshold of octave masking test) wurde schon einmal als Ersatz zum Messen der ohrenharmonischen Schwellen vorgeschlagen. Der Versuch enthält Messungen der Maskierung an einer Oktave oberhalb der Maske (M). Diese Untersuchung wurde zur weiteren Erforschung des TOM in verschiedenen Diagnosen eingeführt. Eine Gruppe von normalen und neuro-sensorisch geschwächten Hörern wurde der TOM Versuch erabreicht: M_f-Bedingungen 500, 1 000, und 2 000 Hz. Die Ergebnisse zeigten statistische (0,05) Unterschiede zwischen den normalen und anormalen Hörer-Gruppen. Ausgezeichnete Zuverlässigkeit zeigte sich bei Wiederversuchen unter allen M -Bedingungen. Vergleicht man die TOM Werte der zwei Gruppen mit den ohrenharmonischen Schwellen der früheren Forschung, so stellt man eine gute Übereinstimmung fest. Somit scheinen die ohrenharmonische Intensität und die TOM Werte die gleichen Information zu liefern. TOM ist aber sehr leicht anzuwenden, verlangt keine komplizierten Apparaturen und bringt ausserdem die Ergebnisse in einer sehr kurzen Zeit. Obwohl weitere Forschung notwendig ist, erscheint dieser Versuch als ein brauchbarer Test in der Audiometrik Diagnose

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Acta Otolaryng 76 426–430 1973

PRESBYACUSIS

VI *Masking of Speech*

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(Received April 9 1973)

Abstract. The effect of age on discrimination of masked speech was studied in white noise at four masking levels, the S/N ratios being +22, +12, +2 and −3 dB. Discrimination of masked speech fell as a function of age the first significant differences from normal being obtained after 40 years of age using S/N ratios of +22 and +12 dB. The age groups over 50 years differed significantly from normal at all levels of noise. The discrimination scores decreased progressively when low S/N ratios were used and with advancing age No significant differences appeared between the right and left ears.

t has been well established clinically that eech discrimination becomes affected with creasing age (Gaeth, 1948 Cawthorne 951 T Palva, 1952 Pestalozza & Shore 1955 Goetzinger et al. 1961 Klotz & Kilbane 1962, König, 1969) This "phonemic regression" (Gaeth 1948) has been explained by the diminished integrative capacity of the aged (Calvi & Finzi 1957) In difficult listening conditions, reduced discrimination of speech becomes more evident

The masking effect of noise and other extraneous sounds depends upon their intensity relative to the intensity of speech, upon their acoustic spectrum and temporal continuity With a decrease in the speech-to-noise (S/N) ratio, the listener's capacity to distinguish phonemic differences decreases and his ability to interpret information becomes poorer Low frequency noise causes more difficulties than high-frequency noise but maximum masking is produced when the noise spectrum mimics the spectrum of speech. Masking by interrupted noise does not equal that of continuous noise until the rate of interruption is about 128 per sec at the frequencies above 500 Hz (T Palva, 1955). Hawkins & Stevens (1950) showed by masking speech with white noise at sensation levels from 10 to 90 dB that speech thresholds were relatively little affected by the lowest levels of masking noise. At higher levels of noise (>20 dB) the thresholds for speech were raised by approximately 10 dB for each 10 dB increment in noise. Curves for thresholds of intelligibility and detectability were approximately parallel.

Simonton & Hedgecock (1953) studied the effect of noise on speech hearing of normal and hard-of hearing individuals using a mixture of white noise and of 60 and 112 Hz pure tones with 98.5 dB overall intensity in a free field. Instead of a fixed level the patient was allowed to choose the speech level he felt was most comfortable In normal ears, using S/N ratios from +3.5 to +9.5 dB the discrimination scores were 78–88% compared with 98–100% in quiet In two presbyacutic cases (65 and 58 years old) the discrimination scores were 58–30% and 66–76% respectively Results in cases with conduction deafness were similar to those in normals.

T Palva (1955) measured speech discrimi-

nation in a 95 dB overall continuous white noise employing a S/N ratio of +10 dB Six subjects with pure presbyacusis all showed lowered articulation scores. The range in quiet was 47–95% as compared with 27–90% in noise. It was suggested that measurement of speech discrimination in noise might be useful in the diagnosis of perceptive deafness.

In the present investigation different masking levels were used to study the changes in speech discrimination in different age groups using young persons as controls.

MATERIAL AND METHODS

The study comprised 120 test subjects, 62 women and 58 men ranging in age from 20 to 87 years. A group of 20 healthy people, 20 to 29 years of age (mean 22 years) formed the main reference group. All other age groups by decades, up to over 70 years, each also included 20 subjects. Mean ages in these groups were 35 44 53 66 and 78 years, respectively

The subjects were free from ear disease other than presbyacusis and there was no history of noise exposure. The routine clinical oto-neurological examination was negative in each case. The pure tone thresholds were measured using the manual technique and the method of limits, from 250 to 8 000 Hz (Madsen Model OB 60 audiometer).

Speech audiometry was carried out with the Madsen Model SU 20 speech unit by the method of T Palva (1952) measuring speech reception thresholds (SRT) and discrimination scores, the latter at a level 30 dB above the SRT Using this same level, speech discrimination scores were then determined in white noise the S/N ratios being +22, +12, +2 and −3 dB The octave sound pressure levels of white noise, using filters with a cut off rate of 40 dB per octave are seen in Table I. Fifteen test words from different lists were delivered to each ear at each level.

The statistical analyses were performed in the Computer Centre of the University of Oulu The Student s *t*-test was used to determine the significance levels.

Table I *The SPL per octave of 65 over all level white noise relative to 0.0002 dyne/cm²*

Centre frequency	125	250	500	1 000	2 000	4 000	8 000
dB	37	41	45	48	53	58	54

RESULTS

The speech audiometric data are seen in Table II The mean pure tone thresholds at frequencies 500, 1 000 and 2 000 Hz were well in agreement with the speech reception thresholds in all age groups. The first significant ($p \leq 0\ 05$) alteration in speech reception thresholds due to presbyacusis appeared in the age group 40–49 years and each subsequent age group differed very significantly ($p \leq 0.001$) from the youngest. There was no significant difference between the right and left ear in any age group.

Speech discrimination without masking in the two oldest age groups was significantly ($p \leq 0.001$) poorer than in the youngest group However discrimination ability was quite

Table II. *The speech reception thresholds and discrimination scores in different age groups*

Age group	Ear	SRT dB	SRT S.D	Discrimination %	Discrimination S.D
20–29	Right	5 7	4 9	98.0	2.8
	Left	6.2	4.2	97 8	2 9
30–39	Right	7.2	5 4	98 0	2.6
	Left	7.8	4.8	97.8	2.9
40–49	Right	8.8	4 8	96.6	4 0
	Left	9.8	5.4	97.2	3.4
50–59	Right	16 0*	10.4	96.5	4 4
	Left	15.0	7 6	95.9	3 9
60–69	Right	23.8	12.8	92.0*	6.7
	Left	24.5	13.0	91.6	9 4
Over 70	Right	33.0*	11.5	86.0	9.2
	Left	32.2	1[illegible]	86.1	10.2

Differences have been calculated relative to age group.
Significance levels. $p < 0.05$,

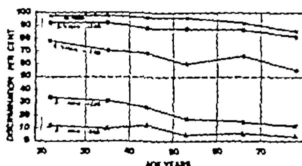


Fig. 1 Discrimination of masked speech in various age groups, with the S/N ratios as parameter

high even in the oldest group only 12% poorer than in the youngest age group There was no significant difference between the right and left ear in any one of the age groups.

The speech discrimination scores at different levels of noise are shown in Fig. 1 and Table III A gradual loss of discrimination appeared with increasing age The first significant ($p \leq 0.05$) difference was noticed between the first and third age group at S/N ratios of +22 and +12 dB Starting from the group 50–59 years, all successive age groups differed significantly from the youngest at all levels f noise. In the oldest age group the average ch score was 11% poorer than in the youngest age group when the S/N ratio was +22 dB Correspondingly it was 24% 23% and 9% poorer when the S/N ratios were +12, +2 and −3 dB. In all age groups, except in the two youngest groups at low masking levels, there was a wide variation of individual discrimination results. No significant right and left ear differences appeared in any one of the age groups.

Comparison between the speech discrimination scores in all age groups without noise and the test using S/N ratio +22 dB showed a statistically significant ($p \leq 0.01$) difference only for the group 20–29 years. From the S/N ratio +12 dB down to −3 dB the discrimination scores were very significantly ($p \leq 0.001$) poorer in all age groups as compared with the discrimination scores without masking.

The discrimination scores obtained at different masking levels in each age group except the groups 40–49 and 60–69 years using S/N ratios +2 and −3 dB differed significantly ($p \leq 0.01$) from one another

DISCUSSION

In the present material balanced to include also younger age groups, the results obtained

Table III *Discrimination scores in speech audiometry at different noise levels*

Age group	Ear	Discrimination, S/N ratio (dB) 22	S.D	+12	S.D	+2	S.D	−3
20–29	Right	92.6	6.4	78.7	11.8	34.4	26.5	
	Left	93.9	6.4	80.2	11.6	37.4	24.1	
30–39	Right	92.6	8.6	70.2	23.8	31.0	23.4	
	Left	90.6	11.6	71.0	26.0	31.0	28.4	
40–49	Right	88.3	17.2	68.7	30.4	26.8	26.8	
	Left	87.9*	11.1	67.6	20.8	23.4	.5	
50–59	Right	88.0	1..4	60.4	25.5	17.0	19	
	Left	84.6	18.4	63.6	27.4	21.8	20	
60–69	Right	87.0	12..	66.6	22.5	15.0	19	
	Left	83.6	16.8	66.8	20.6	14.8	1	
Over 70	Right	81.4	14.1	55.2	20.2	11.7	15	
	Left	79.7	13.7	52.8	14.7	7.4	1	

Differences have been calculated relative to the youngest age group.
Significance levels. $p < 0.05$ $p < 0.01$ $p < 0.001$

in presbyacusis by pure tone and non-distorted speech audiometry were similar to those reported earlier (T. Palva, 1952; Pestalozza & Shore 1955; Goetzinger et al. 1961; A. Palva & Jokinen, 1970). The speech reception thresholds became significantly poorer than normal in the same age range, the fifth decade, in which there was a distinct lowering of pure tone thresholds due to presbyacusis in the area 500–2 000 Hz (A. Palva & Jokinen, 1970). Lowered discrimination scores were *found only in the groups over 60 years of age.*

Comparison of speech discrimination without and with masking showed that when the S/N ratio was reduced to +12 dB the presence of noise caused a significant reduction of articulation scores. This of course increased to large differences from normal when using smaller S/N ratios.

In noise, a significant lowering in discrimination from normal (youngest age group) occurred from the age groups 40–49 years onwards using the S/N ratios +22 and +12 dB. With S/N ratios +3 and −2 a significant reduction from normal was not evident until from the age of 50 upward.

The present discrimination scores are not quite comparable to those reported by Simonton & Hedgecock (1953) since their noise was not thermal but had a peak at 112 Hz. This apparently was the reason why their figures at S/N ratios +3.5 were still around 80% while the present scores at S/N ratio +2 were around 25%. The results obtained by T. Palva (1955) in his 6 subjects exceeding 60 years of age were in the same range as reported here.

Quite recently Morales-Garcia & Poole (1972) carried out speech tests in white noise at S/N ratios +10, +5, 0 and −5 upon normal subjects and patients with a variety of intracranial lesions. In the normals the average discrimination values were 79%, 67%, 43% and 20% respectively. These figures agree reasonably well with the present normative data, which were 79%, 36% and 15% at S/N ratios +12, +2 and −3. In patients, with brain stem and temporal lobe lesions Morales-Garcia & Poole noticed significantly lower than normal masked speech scores. A similar phenomenon was noted in this study in presbyacusis. It is apparent that the central auditory pathways of old people are subject to changes that are responsible for the impaired discrimination even though localization of the alterations to different levels of the central auditory pathways or to the auditory cortex cannot be established by this test.

In the filtered speech test, discrimination *dropped earlier as a function of age than using* the present test. A. Palva & Jokinen found, using both the monaural and Matzker's binaural test, that the speech scores already began to fall in the fourth decade and that there was asymmetry in the age groups over 60 years. The latter was attributed to the effect of cerebral dominance becoming evident during the degeneration of the central auditory pathways. The present test demonstrated no significant right and left differences at any masking level, which is in agreement with findings of Morales-Garcia & Poole in patients with unilateral right or left temporal lobe lesions. It is possible that this difference between the masking test and filtered speech test may be due to the greater sensitivity of the latter because filtration selectively removes some of the important formants. In clinical use the masked speech test is not much simpler however and therefore further work on different patient groups seems indicated to reveal its value as a part of the diagnostic test battery.

ZUSAMMENFASSUNG

Die altersbedingte Herabsetzung der Diskrimination der maskierten Sprache wurde im weissen Lärme mit vier Sprach-Lärm-Proportionen +22, +12, +2 und −3 dB untersucht. Die Verständlichkeit der maskierten Sprache wurde mit zunehmendem Alter schlechter. Im Alter von über 40 Jahren wurde eine bedeutsame Herabsetzung der Diskrimination im Vergleich zur Altersgruppe von 20–29 Jahren mit Sprach-Lärm-Proportionen +22 und +12 dB festgestellt. Die m kierten Sprachwerte im Alter von über 40 Ja bei allen Sprach-Lärm-Proportionen waren d niedriger als die der Jüngeren. Die Dis

wurde mit zunehmenden Alter und niedrigen Sprach-Lärm-Proportionen progressiv schlechter. Die Vergleiche der Werte der linken und rechten Ohren liess keine signifikante Differenz erkennen.

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Acta Otolaryng 76 431–437 1973

THE HISTOLOGICAL EFFECT OF AN ADHESIVE IN THE MIDDLE EAR

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(Received April 17 1973)

Abstract. Small amounts of the adhesive Histoacryl® were placed in the middle ear of thirteen guinea pigs. Lightmicroscopic examination of the temporal bones at 2 hours to 19 weeks after application showed initially partial destruction of the mucous membrane and periosteum of the promontory; varying degrees of osseous destruction and inflammation occurred until 7 to 8 weeks; then reparative processes came into the picture, occasionally with giant cells surrounding the adhesive material, which was present in the middle ear even 19 weeks postoperatively. Lightmicroscopic studies revealed no alterations in the inner ear. The pronounced reparative processes accompanied by new formation of connective tissue presumably are responsible for the prolonged bondage action. Our experiments, which are in accord with previous reports on the clinical application of adhesives, suggest that histoacryl may be used in ossiculoplasty when applied in small amounts and thinly spread.

The need for sutureless repairs of body tissues was recognized as early as 1791 by John Hunter who wrote. "The passage of the needle and ligature must always produce suppuration through the whole passage. The use of a sticking plaster would be more general in its application." However it was not until the first liquid plastic adhesives were developed in the 1960s that surgical repair of various tissues of the body without the use of sutures was possible.

A large number of papers on experimental and clinical application of various adhesives have been published in recent years. The need for non-suture repair has been especially urgent in vascular surgery such as small-vessel anastomoses, ductal repair of the ureter and bile duct, stump closure of the bronchus and intestinal anastomoses.

Also in ocular surgery have adhesives been useful. Blomfield et al. (1963) found that methyl 2-cyanoacrylate could be safely employed, particularly subconjunctivally although, as stressed by Ellis & Levine (1963) certain precautions are called for since the use of large amounts may cause inflammatory reactions.

Studies of polymers as carcinogenic agents in animals by Oppenheimer et al. (1955) showed methyl-acrylate to be only half as carcinogenic as silastic film, which has been widely used in tympanoplasty A few studies on the use of adhesives in tympanoplastic surgery have been published, the first one was by Watson & Maguda (1965), who performed myringoplasty on a total of 25 tympanic perforations in cats. They concluded that a rapidly polymerizing adhesive might be a useful adjunct in myringoplasty

Using methyl-acrylate, Elbrønd (1970) conducted an experiment in reconstruction of the osseous chain on temporal bone preparations. He found the agent to be useful, however inapplicable in vivo because of its toxicity as demonstrated by Kerr & Smyth (1970). Other workers however have used methyl-2-cyanoacrylate in tympanoplastic surgery with good clinical results and without signs of toxic (de Sa, 1969) and after the

a longer side chain such as in iso-butyl-cyano-acrylate and butyl-organoacrylate the toxic reaction in the tissue seems to be less pronounced (Healey et al. 1965). These compounds have been used in numerous tympanoplasties, and so far no clinical damage has been reported (Surjan & Bodi, 1971; Gerlach, 1971; Decher 1971; Sørensen 1971; Schnieder 1972).

In 1959 Sataloff reported favourable results from ossiculoplasty with ostamer, which is a polyurethane foam.

Closures of liquor fistulas to the nose and ears have been performed too by the use of adhesives (Pfalz, 1969).

It has been emphasized that acrylate should not be applied on the promontory or in the region of the round and oval windows, and that the adhesive is most effective and least toxic when used in small amounts and thin layers, whereas application in thick layers results in tissue damage and bondage failures.

Histological studies of tissue reactions elicited by adhesives

The histological reactions to the compounds that are applicable as adhesives have been studied in cell cultures (fibrocytes and leucocytes) (Hulliger 1962) as also in various tissues in animal experiments (Reynolds et al. 1966). Methyl acrylic acid has no inhibitory effect on cell cultures, however in the liquid form it has showed a cell toxic effect that is thought to be attributable to the content of free monomers. On the other hand, no noxious effects seem attributable to the evolution of heat when small amounts of the adhesive have been used. Intramuscular implantation of butyl-2-cyanoacrylate in guinea pigs caused definite proliferation of the surrounding connective tissue after 7 days (Saeger et al. 1972). Proliferation went on until the 65th day. Giant cells were seen, which contained up to 20 nuclei, and leucocyte infiltration with numerous eosinophils could be demonstrated in the tissue by means of nile blue and ORO-staining up to the 95th day. The elimination rate has also been assessed by implantation of radioactive methyl-acrylate. By this method Reynolds et al. (1966) found that acrylate in skin incisions had practically disappeared from the wound after 16 days. All radioactivity had ceased after 64 days.

This rather severe tissue reaction is felt to be responsible for the permanent bonding action.

Tabb (1968) applied isobutyl-2-cyanoacrylate monomer on the ossicles within the tympanum and on the adjacent bone of four guinea pigs and found no evidence of tissue toxicity or inflammation.

In a histologic study of cats Kerr & Smyth (1971) however found pronounced toxic response to butyl-acrylate. Not only were the middle ear structures severely damaged, but also the inner ear was affected, even though only a small amount of adhesive had been placed on the stapes footplate. They felt that the use of plastic adhesives should be avoided, because of the risk of damage to the middle and inner ear.

The purpose of the present study was to evaluate, by further histologic studies, the toxic effect of butyl-acrylate when applied to the middle ear of guinea pigs.

MATERIAL AND METHODS

Thirteen young, healthy guinea pigs with a positive Preyer-reflex were used; none had shown evidence of otitis media on otoscopy.

Following anesthesia with pentymal i.p., one drop of Histoacryl® was injected under operating microscope through the posterior part of one ear drum, using a syringe and a fine needle. In 10 guinea pigs the other ear was left untouched for control purposes. In the remaining 3 animals puncture of the posterior part of the ear drum was performed with a fine needle at a later time, but without injection of adhesive.

Two animals were found dead 2 or 3 hours after the anesthesia. One was found in opis-



Fig. 1. Immediate effect of histoacryl. The adhesive is distributed over the osseous surface; the mucous membrane and the periosteum being partially destroyed. ×360.

thotonus 1 1/2 weeks postoperatively and was then killed. Another was found dead after 4 1/2 weeks following poor general condition and diarrhea for about 1 week. The remaining guinea pigs showed no evidence of poor health. They were sacrificed at intervals from 1 to 19 weeks. The temporal bones were removed, fixed in Lillie's fluid, using 2% glutaraldehyde, then decalcified in a 5–10% EDTA solution. When X-rays showed complete decalcification, the specimens were embedded in paraffin wax, sectioned and stained with haematoxylin-eosin and PAS.

RESULTS

The immediate effect of histoacryl could be observed in 2 animals that died during anesthesia a few hours after the material had been placed in their middle ears.

Examination showed alterations in the periosteum, which in the region of the promontory was partially destroyed, irregular with festoon-like formations. At this stage no cell infiltration was present in the tissue and there was no pus in the middle ear.

In the 2 animals that died 1 1/2 and 4 1/2 weeks postoperatively the examination revealed a severe tissue reaction with infiltration of granulocytes and pus in the middle ear.

In 3 animals killed after 7, 7 and 8 weeks respectively there were small destructions of the bone adjacent to the promontory, where histoacryl was seen embedded in granulation tissue and surrounded by giant cells. Also the handle of the hammer had been partially destroyed. There were early reparative processes with newly formed connective tissue and adherence between the ear drum and the promontory. Only in one animal was a little pus found in the middle ear.

After 11, 12 and 13 weeks (3 animals) definite reparative processes were present, including connective tissue formation over the promontory, superficial osseous destruction of the tympanic capsule and the handle of the hammer, but no pus. In 1 animal the middle ear was normal, with no histoacryl left.

The 2 animals that survived the lo
and 19 weeks) had superficial
the tympanic capsule over the

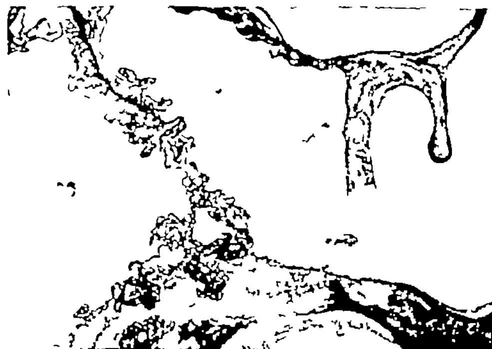


Fig 2. Widespread osseous destruction of the promontory. Bone tissue has been replaced by connective tissue leaving only a thin osseous rim. 4 / weeks after application. × 73

where histoacryl was found embedded in the granulation tissue and giant cells. Not even in those animals was there any pus in the middle ear

In none of the animals were histologic al ations observed in the inner ear The spiral ligament and the organ of Corti showed no evidence of pathologic change Destructions in the region of the windows were not observed

DISCUSSION

The application of histoacryl in the middle ear caused, as an immediate reaction super ficial lesions to the mucous membrane and the periosteum as could be observed in the 2 animals dying during anesthesia (Fig 1) The lesions may be caused by severe dehydra tion during the polymerization of histoacryl, whereas burns due to heat developing in the course of that process could be excluded. We measured the temperature in one drop of histoacryl during polymerization and found no changes exceeding 1 C.

Occasional inflammatory alterations with infiltration of granulocytes in the tissue and purulent secretion in the dle ear developed. This reaction may h primary lesion, or it m tion of histoacryl the l ever being less likely si subsided after 7 to 8 w histoacryl was still pre terial inflammation c procedure seems unl three control perfo with otitis.

As the inflamma processes with new tissue occur and ably contribute to material, are prese and fibrous adh drum and the midd

Even after 19 in the middle ear to progress essent ample in skin inc



Fig. 3 Partial osseous destruction. The histoacryl is surrounded by newly-formed connective tissue, indicating reparative processes. 7 to 8 weeks after application. 475.

terial has disappeared after 16 days, whereas it may be found in muscular tissue up to the 95th day (Saeger et al. 1972)

Bone necrosis, which occurs at the site of the primary lesion in the periosteum, seems to reach its maximum within 10 to 12 weeks, whereafter proliferation of connective tissue in the defective bone is apparent. This is most pronounced in the specimens that contain considerable amounts of histoacryl, and in one case (Fig. 2) the destruction involves the entire thickness of the labyrinthic capsule. Even in this case, however no histologic changes are detectable in the cochlea.

In accord with previous experiments we find the damage to be most extensive where histoacryl has been applied in large amounts, whereas only a moderate reaction and negligible necrosis is observed where small amounts have been used.

Our experiments do not allow of evaluation of the bondage action however the adherences found between drum, hammer and the promontory support the assumption that the tissue reaction contributes to permanent bonding (Saeger et al. 1972). Such an effect can only be achieved if the material is resorbed slowly enough to allow the formation of connective tissue before the adhesive has disappeared. The inflammatory reaction must be moderate enough to ensure that destructions and necroses do not prevail before the onset of the organization of granulation tissue.

The ideal adhesive must possess the following qualities:

1) great immediate stickiness,
2) slow absorption,
3) an ability to produce a limited matory reaction followed by prc



Fig. 4. Giant cells in granulation tissue close to the adhesive. × 635.

connective tissue but without pronounced ...osis and secondary infection
... no absorptive toxicity.

The lesions and subsequent reactions elicited by small amounts of histoacryl applied in thin layers do not, in our opinion, justify the conclusion that plastic adhesives should be abandoned. In the clinical use in tympanoplasties complications that could be attributed to histoacryl have in fact never been reported in the literature.

Because of its toxic effect we do however recommend that histoacryl be applied only in small amounts and in thin layers, and application near the window should be avoided.

ZUSAMMENFASSUNG

Kleine Mengen eines Gewebeklebers (Histoacryl®) wurden in den Mittelohren von dreizeh... Meerschweinchen appliziert. Nach zwei Stunden bzw. bis um neunzehn Wochen wurden die Tiere getötet. Die Schläfenbeine wurden lichtmikroskopisch untersucht. Im Anfangsstadium wurde eine teilweise Zerstörung der Schleimhaut und des Periosts von Promontorium gefunden. Nach sieben bis acht Wochen wurden verschiedene Grade von Knochenzerstörung und Entzündung gesehen. Danach dominierten die Wiederaufbauprozesse. Von Riesenzellen und Bindegewebe umgegeben wurde der Klebstoff an vielen Stellen wiedergefunden. Sogar noch neunzehn Wochen nach der Applikation wurde der Klebstoff im Mittelohr nachgewiesen. Lichtmikroskopisch wurde keine Zerstörung des inneren Ohres entdeckt. Für die fortdauernde Klebrigkeit sind ohne Zweifel die Wiederaufbauprozesse mit ihrer Neubildung von Bindegeweben verantwortlich. Unser Versuch bedeutet, dass in Übereinstimmung mit Ergebnissen früherer klinischer Untersuchungen Histoacryl in der Ossikuloplastik angewandt werden kann, wenn es nur in kleinen Mengen und in dünnen Schichten appliziert wird.

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Acta Otolaryng 76 438–442, 1973

THE OTOLITH ORGANS AND THE NYSTAGMUS PROBLEM

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(Received February 28 1973)

Abstract The material consisted of 35 cats which were divided into two groups. In one group a selective bilateral sectioning of the horizontal ampullar nerves was performed. Nystagmus did not occur in these animals. Tilting in either direction never provoked nystagmus. In the other group unilateral selective sectioning of the utricular nerve was performed. In all the animals a horizontal spontaneous nystagmus developed directed to the nonoperated ear. Tilting toward the operated ear increased the nystagmus frequency whereas tilting in the opposite direction inhibited nystagmus. It was concluded that the utricle in itself never gives rise to nystagmus, but unilateral damage may readily induce a nystagmus that can be modulated by alterations in the influence of the g-forces. This is owing to the intimate collaboration between the utricles and the semicircular canals.

The question whether the otolith organs can induce nystagmus has long been discussed on the basis of both clinical and experimental investigations. This is an important problem especially as it is intimately related to the problem of the so-called positional nystagmus. As early as 1921 Bárány (1921) assumed that positional nystagmus might result from damage to the otolith organs. Nylen (1950) considered that positional nystagmus was the result of deficient interplay between the otolith organs and the semicircular canals. Cawthorne & Hallpike (1957) found pathologic changes in the utricular macula in a patient who had earlier suffered from positional nystagmus.

Several authors have made animal experiments in order to study this problem. Maxwell (1923) found that mechanical pressure on the otolith organs did not cause any nystagmus. Nor could Versteegh (1927) Ulrich (1935) and Szentagothai (1952) elicit nystagmus when using the same technique. Versteegh (1927) Sullivan et al. (1957) Janeke (1968) and Janeke et al. (1970) were unable to show any nystagmus after sectioning the utricular nerve. Graybiel et al. (1952) and Bergstedt (1961) failed to elicit nystagmus on applying linear acceleration.

On the other hand Fernandez et al. (1959) succeeded in producing nystagmus by sectioning the utricular nerve in the cat. Owada et al (1960) elicited nystagmus by mechanical pressure on the utricle of rabbits. By applying linear acceleration McCabe (1964) was able to induce nystagmus in both animals and man. Niven et al. (1966) elicited nystagmus in man by using linear acceleration.

Thus, opinions have hitherto differed although many authors are aware that there must be some kind of interplay between the semicircular canals and the otolith organs. Caston (1968) also found that postrotatory nystagmus diminished in the frog after sectioning the utricular nerves. Jongkees & Philipszoon (1964) have shown on unilabyrinthectomized cats that tilting inhibits nystagmus. Quite recently Fluur & Siegborn (1973) have demonstrated in a number of experiments on animals that there is a very intimate collaboration between the utricle and the semicircular canals.



Fig. 1 Consecutive curves from a cat after selective unilateral sectioning of the left utricular nerve followed by tilting. Upper curve shows electronystagmography and lower curve the tilting of the table. Tilting to the right upwards, to the left downwards.

Fluur & Mellström (1971) have shown that electrical stimulation of the lateral part of the utricular surface can in certain cases elicit nystagmus in an ipsilateral direction. These findings have stimulated us to study this question more closely in order to try to throw some light on the problem of positional nystagmus.

MATERIAL AND METHODS

Thirty-five adult cats were used for the experiments, which were divided into two groups.

1. Bilateral selective sectioning of the horizontal ampullar nerves, followed by tilting around the cat's longitudinal axis (5 cats).
2. Unilabyrinthine selective sectioning of the utricular nerve followed by tilting around the animal's longitudinal axis (30 cats).

A vertical incision, 3 cm long, was made immediately anterior to the external ear. The cartilaginous external meatus was dissected free and cut close to the temporal bone, whereafter it was attached to the side by a self-retracting forceps. The middle ear was explored, and the malleus, incus and the tensor tympanic muscle were removed. The facial canal was opened, and part of the nerve resected. At the bottom of the canal two small blue spots were visible which marked the ampullae of the horizontal and the anterior vertical canals. Medial to these, a fenestration was performed which was large enough for the visualization of the nerves from the two ampullae and the utricles. The horizontal ampullar nerves or the nerve from the utricle were cut as far away as possible from the other nerves. Great care must be taken not to damage the membranous labyrinth. Under the microscope, however it was not so difficult, since it was possible to see all the other structures and their borders, and afterwards to check by visual inspection.

The eye movements were recorded partly by direct visual ocular inspection of the cat's eyes, and partly by traditional electronystagmography with needle electrodes in the lateral canthi bilaterally and with the ground electrode in the skin of the neck.

Stimulation was induced by tilting the animals 60 degrees around their longitudinal axis to either side.

RESULTS

After bilateral sectioning of the horizontal ampullar nerves, none of the cats had any nystagmus at all when wakened to a level of superficial anesthesia. Tilting in either direction caused a slight eye deviation in the direction of tilting in only 2 cats, but no nystagmus occurred in any of th

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frequency from 5 to 20 beats per 10 sec. Tilting toward the non-operated ear resulted in a total inhibition of nystagmus in 15 cats. In 6 cats an initial increase in frequency from 5 to 18 beats per 10 sec, was subsequently followed by total inhibition In 9 cats there was only a decreased frequency but no total inhibition If instead, the animals were tilted toward the operated ear 19 cats showed a clear increase in nystagmus frequency with 2–12 beats per 10 sec (Fig 1) In 11 cats no appreciable alteration in nystagmus was observed.

DISCUSSION

In the introduction it was mentioned that one group of investigators induced nystagmus by subjecting the otolith organs to different kinds of stimuli, whereas another group who used approximately the same technique failed to provoke any nystagmus at all. The present investigation has shown that both groups can be partly right For the investigation has demonstrated that, in experimental animals without horizontal semicircular canals, no horizontal nystagmus can be induced by tilting which stimulates, or inhibits the sensory cells in the area of the utricular surface that has provoked horizontal eye movements. For nystagmus can only be induced from the semicircular canals, and if these are lacking, impulses no longer occur in the reflex arc which can be modulated by the utricle

On the other hand, selective unilateral utricular damage can very readily cause both spontaneous and positional nystagmus. This is not because the utricles, in themselves, can induce nystagmus but because all the sensory organs in the vestibular apparatus are differential organs (Fluur & Mellstrom 1971) and moreover the otolith organs and the semicircular canals interact very intimately (Fluur & Siegborn, 1973) In other words, the vestibular apparatus is a very complex computer in which the slightest difference in activity in one of its sensory organs immediately influences the activity of the other organs, and

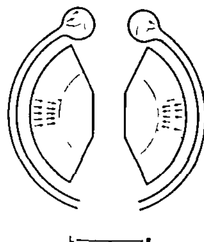


Fig 2 Schematic picture of utricles and horizontal semicircular canals in resting position. Arrows indicate orientation of hair cells, i.e. the direction in which they increase their discharge frequency

secondarily also the activity of the effector organs—in this case the oculomotor system—will be modulated

Referring to earlier neurophysiological experiments concerning utricular stimulation and oculomotor reactions (Fluur & Mellström, 1971) we shall try by using a few pictures to explain what happens during the tilting of the animals. At rest there is a balance of activity both between the two utricles themselves and between the two horizontal semicircular canals themselves. Furthermore the

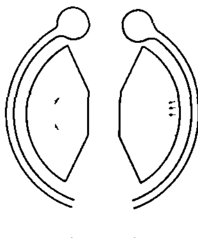


Fig 3 Schematic picture of utricles and horizontal semicircular canals in resting position after [illegible] sectioning of left utricular nerve. Arrows [illegible] same as in Fig. 2. Number of arrows related to discharge frequency

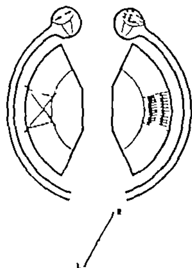


Fig. 4. Schematic picture of utricles and horizontal semicircular canals after selective sectioning of left utricular nerve, followed by tilting to the left. Arrow indications as in Fig. 2. Number of arrows symbolizes discharge frequency

activities between the utricles and the semicircular canals are balanced (Fig. 2). Sectioning the utricular nerve on one side immediately disturbs this balance. The utricle normally facilitates the slow phase of nystagmus *from the ipsilateral horizontal semicircular* canal (Fluur & Siegborn, 1973). By sectioning the nerve this positive triggering becomes inoperative. Because of the differential reaction (Fluur & Mellström, 1971) the activity of the contra-lateral utricle and of the horizontal semicircular canal now predominates, and this *causes spontaneous nystagmus toward the non-*operated ear (Fig. 3) This nystagmus can now be modulated by tilting. If the animal is tilted *toward the operated ear (Fig. 4) the activity* in the normal utricle intensifies, which in its turn facilitates the slow phase, elicited from the ipsilateral horizontal semicircular canal and simultaneously inhibits the activity from the horizontal semicircular canal of the operated ear (Fluur & Siegborn, 1973). This causes the nystagmus frequency to increase, just as the experiments have shown.

If instead, the animals are tilted toward the non-operated ear (Fig. 5) the activity of the normal utricle slackens and no longer facilitates the nystagmus from the ipsilateral semicircular canal. Simultaneously the antagonistic oculomotor muscles are no longer inhibited and the nystagmus disappears.

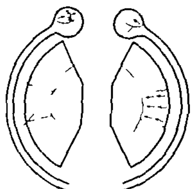


Fig. 5. Schematic picture of utricles and horizontal semicircular canals after selective sectioning of left utricular nerve, followed by tilting to the right. Arrow indications as in Fig. 2. Number of arrows symbolizes discharge frequency

ZUSAMMENFASSUNG

Das Material besteht aus 35 Katzen, die in zwei Gruppen eingeteilt wurden. In einer Gruppe wurde eine selektive Abtrennung des N. ampullaris canalis horizontalis bilateral vorgenommen. Keines dieser Tiere erhielt Nystagmus. Kippung des Tieres zur Seite ergab niemals einen Nystagmus. In einer anderen Gruppe wurde selektiv eine unilaterale Abtrennung des N. utricularis vorgenommen. Alle diese Tiere erhielten einen horizontalen Spontannystagmus zur Seite des nichtoperierten Ohres hin. Kippung zur operierten Seite hin resultierte in einer Steigerung der Nystagmusgeschwindigkeit, während die Kippung zur anderen Seite den Nystagmus inhibierte. Die Folgerung war, dass ein Utrikulus niemals per se Nystagmus ergeben kann, aber dass eine unilaterale Schädigung sehr wohl einen Nystagmus induzieren kann, der bei veränderterem Einfluss der g-Kräfte moduliert wird. Das hängt von der Zusammenarbeit zwi Utrikulus und den Bogengängen ab.

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ON LINE ANALYSIS OF OPTOKINETIC NYSTAGMUS BY SMALL GENERAL PURPOSE DIGITAL COMPUTER

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Abstract A program for a small general purpose digital computer was developed to analyze electrooculograms of optokinetic nystagmus in monkeys. It provides immediate print-out of means and standard deviations of maximum velocity of fast phases, velocity of slow phases, amplitude, total deviation during slow phases and number of nystagmic beats occurring in 15 sec trials. It has also the potential to measure other response features and the constants can be easily changed for application to individual circumstances. The validity of the system was verified by both simulation and sample analysis. The small size and relatively low cost of the equipment makes the system ideally suited for "on-line" close-loop experiments in the physiologic laboratory and for rapid quantification of data in the clinical setting which would allow evaluation of normal and abnormal eye movements under a variety of conditions within the same session.

Electrooculography (E.O.G.) has become the routine method for the clinical and experimental evaluation of eye movements. This technique utilizes the polarized electric field (positive in front and negative in the back) of the eye globe. As the eye moves, the distribution of electric potentials in the surrounding tissues changes accordingly. It has been shown that the changes detected by electrodes placed in the periorbital area on each side of the eye are proportional to the amount of displacement of the globe (Leksell, 1939). This relationship is linear up to 35° to each side of the midposition and can be recorded with little or no distortion by the use of d.c. amplifiers. The time base of the recording apparatus and the amplitude of the potential provide a graphic representation of the angular position of the eye as a function of time. It is relatively simple thereafter to determine the velocity of the eye movement as the first derivative of eye position.

A phenomenon that has been extensively studied with this method is optokinetic nystagmus (O.K.N.), i.e. the pattern of eye movements elicited by successive moving stimuli traversing the visual field. The fully developed response consists of relatively slow ocular deviations in the direction of the moving stimuli alternating with relatively fast eye movements in the opposite direction. In the past, methods have been developed to analyze electrooculograms of nystagmus by means of large scale digital computers (Herberts et al. 1968 Honrubia et al., 1971) or to provide only a nearly continuous record of slow phase velocity (Tole & Young, 1971). The purpose of the present communication is to describe a system for the analysis of O.K.N. requiring only standard recording equipment and a relatively inexpensive, small general purpose digital computer. Although our specific application has been to explore the effect of stimulus parameters and various brain lesions on the characteristics of the optokinetic response in the key the method can be utilized as study of normal and abnormal ey in man.

This work was aided in part by U.S.P.H.S. Grants (MH-02261 and K3-EY 16,865).

METHODS

Experimental set-up and equipment

The experimental subjects were 6 normal monkeys (*Macaca mulatta*). The animal was restrained in a "primate chair" with the head held in position via three machine screws chronically implanted in the skull and fixed to the chair through ball-joints (Bossom 1964) The stimulation equipment has been previously described in detail (Valciukas, 1972 Valciukas et al. in press) In short, a 106 × 106 cm rear projection screen was mounted perpendicular to the monkey s primary line of gaze at a distance of 25 cm from the eyes. A specially constructed 35 mm film strip projector was used to project 5 luminous vertical stripes, 2 cm in width, separated by dark bands of 19 cm This pattern was moved from right to left or vice-versa over a wide range of constant velocities by means of a servomotor coupled to the film transport mechanism. Such stimulation elicited O K.N which as is well known, showed a progressive increase in the maximum slow phase velocity for several seconds. Therefore it should be emphasized that meaningful statistics for slow phase velocities could only be obtained from taken after the response had fully

A silver-silver chloride recording electrode was chronically implanted at each lateral canthus. These were connected subcutaneously to a plug anchored to the skull. Amplification of potentials was obtained via a low level DC preamplifier (Grass 7P1 A) and a driver amplifier (Grass 7DA B). An ink writing polygraph (Grass model 7) produced a permanent record of the activity Polarities were selected so that eye movements to the right or to the left resulted in upward or downward moving signals respectively One of the outputs of the driver amplifier was modified via an operational amplifier to match a 10-bit analog to-digital converter (Digital Equipment Corp. PDP ADO8) which in turn was connected to a small general purpose digital computer (PDP 8/I) with 4096, 12 bit words and 1.5 μsec cycle time The output device was a standard teletype (ASR 33) capable of printing 10 characters per sec.

Program theory

The principle of the method is illustrated in Fig. 1 in terms of an idealized nystagmic beat consisting of a rapid eye movement to the right (up-going signal) of 45 msec duration followed by a slow eye movement to the left (down-going signal) of 420 msec duration. For a well established O K.N response there is usually about a 10 1 ratio between the velocities of fast and slow phases. The graph can be conceived as a Carthesian system with the abscissa as time (t) and the ordinate voltage which in turn is linearly related to eye position (p). If the values of p_1 and p_2 at times t_1 and t_2 are recorded the difference $\Delta p = p_2 - p_1$ would be of positive sign in this example of a movement toward the right. Conversely for the eye movement to the left $\Delta p = p_4 - p_3$ at t_4 and t_3 would be of negative sign.

The computer technique is quite straightforward. The analog signal, i.e the actual voltage of the electrooculogram, is fed into th analog to-digital converter (ADC) On command from the program, the ADC assigns a numerical value proportional to the voltage of the incoming signal. This value p_1 is temporarily held in the computer memory At a fixed time interval later (Δt) this process is repeated and a second value p_2 is obtained. The difference between p_2 and p_1 is calculated (Δp) and stored. The computer proceeds to collect a consecutive series of Δp values for successive Δt intervals. The expression $\Delta p/\Delta t$ is the first derivative of the input signal, i.e. its velocity Since Δt is constant Δp is directly proportional to the velocity A Δt value of 15 msec is chosen because it provides about 5 Δp determinations for a typical rapid eye movement with actual numerical values high enough to minimize the effect of electric noise in the recording apparatus and the ADC.

A Δp "discriminator" magnitude is selected

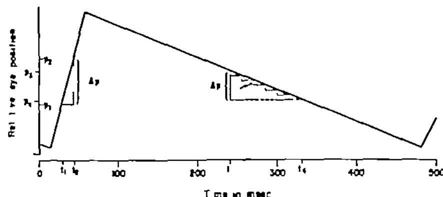


Fig. 1 Idealized nystagmic beat with a fast phase going upward (to the right) followed by a slow phase going downward (to the left). Note that a 15 msec interval between t_1 and t_2 provides p_1 and p_2 values for the fast phase, giving a positive Δp of adequate magnitude. For the slow phase, it is apparent that a 15 msec time interval would provide an extremely small Δp (*arrow*) and consequently it is necessary to summate the Δp values for 6 consecutive 15 msec intervals (i.e. 90 msec represented by t_3 and t_4) so as to obtain a sufficiently large value of Δp to separate it more effectively from the noise in the system. This manipulation leaves the stored Δp values of the fast and slow phases on a different time base. To compensate for this, the highest Δp of each fast phase is multiplied by 6 prior to calculation of mean maximum velocity of fast phase and its standard deviation.

so that eye movements are treated as fast or slow depending on whether the Δp is above or below this value respectively When Δps are above the "discriminator" level the values are stored. When Δps are below the "discriminator" level, an additional step is necessary because the magnitude can be so low that the signal may not be reliably distinguished from the noise in the system. This difficulty is overcome by summating 6 consecutive such Δp values which in effect is equivalent to an increase of Δt to 90 msec (see Fig. 1). In order to maintain a consistent time base for velocity measurements, the Δp values of fast phases are multiplied by 6 prior to print-out (see below).

Program operation

The program used to collect, store and analyse the O.K.N elicited during 15 sec trials consists of an "on-line" and "off line" portion. Fig. 2 is a flow chart of the "on line" segment which calculates 1 000 Δp values for successive 15 msec intervals of the electro-oculogram. This number of differences accounts for 15 sec of O.K.N It determines, by comparison with the Δp "discriminator" level (see above) whether each Δp is part of a fast or a slow phase, and by the sign of the values, whether the eye movement is to the right or to the left. In this process, each Δp is coded accordingly The coding technique takes advantage of the fact that the computer has a 12-bit memory word, whereas the ADC is a 10 (0–9) bit device with bit 9 being the least significant. By clearing this bit, the last octal number (bits 9 10 and 11) of the memory word is left free, and can consequently be used to code the p. The numbers used for this purpose are Φ for fast to right, 1 fast to left, 2, slow to right 3 slow to left. The program stores in a table (Fig. 3) the Δp values coded as Φ or 1 for above the "discriminator" level. When a value is below this level, the first Δp is temporarily held while subsequent Δps are obtained and compared to the "discriminator" value. If 6 successive Δps are below this level, the difference between the last and first Δp values of that series is stored in the table coded as 2 or 3 (Fig.
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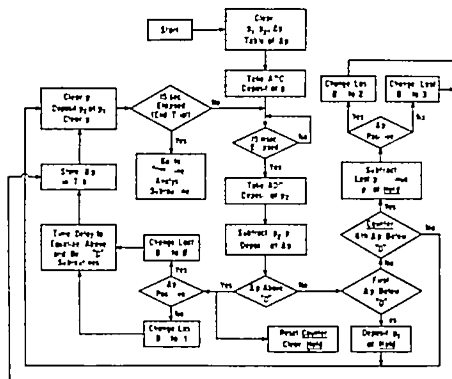


Fig. 2. Flow chart of program. "D" indicates "discriminator" level. The 15 msec intervals are obtained by repetition of a fixed duration program loop. The 15 sec interval is derived by counting 1000 consecutive 15 msec intervals.

At the end of the 15 sec period of collection, the program shifts to the "off-line" segment which analyses the table of differences by means of the arithmetic routine (PDP Floating Point Package) and prints out the results in decimal numbers. A subroutine is available to type out the entire table of Δps in octal numbers (Fig 3) only when there is a need for it because it requires about 3 minutes of printing time. The "off line" analysis proceeds as follows:

Fast phases to the right. The program searches the table for the first series of numbers ending in 0, summates them and stores the result as the *amplitude* of that phase. The largest of these Δp values in the series is identified, multiplied by 6 to correct for the 6:1 ratio of the Δt used for fast and slow phases, and stored as the *maximum velocity* for that phase. The program then finds the second sequence of numbers ending in 0 and repeats the process, adding the maximum velocity and amplitude values of the second fast phase to those obtained for the first. This procedure continues until all of the series ending in 0 have been treated similarly for the entire table. Subsequently the sum of maximum velocities is divided by the number of such values (N) and printed out as the mean maximum velocity (MMV) of the fast phases to the right. The sum of the amplitudes is also divided by N to give the mean amplitude (MA). For each of these mean values, the standard deviation (S.D.) is calculated and printer. As a final step N is provided as the number of fast phases which is usually equal to the nystagmic beats occurring in the 15 sec trial.

Fast phases to the left. The identical process described above is utilized except that it searches for the sequences of numbers ending in 1.

Slow phases to the right. This subroutine searches the table for all numbers ending in 2, obtains their sum (TD) and divides it by the number of such values. The result is printed out as the mean velocity of the slow phases (MV) to the right, along with the standard deviation and the TD value which represents the total deviation of the eyes during these slow phases in the 15 sec trial.

Slow phases to the left. These values are

obtained by the same subroutine as above, except that table numbers ending in 3 are utilized.

Simulation analysis

A triangular wave form generator was used to test the ability of the program to obtain Δp values and to demonstrate the linear relationship between the slope of the input and the magnitude of the output. A 15 sec record was made for each of 17 frequencies. The computer output of mean maximum velocity was obtained for seven values between 30 and 153 Hz since these generated slopes e al to or greater than a usual fast phase of C K N The mean velocity was obtained for 10 values between 2.87 and 26 Hz since these produced slopes equal to or slower than the usual slow phases. The graphic display of the results demonstrated a strict linear relationship between frequency (slope velocity) and the output of the computer for both the above (MMV) and below (MV) "discrimina o level subroutines.

Sample analysis

An example of the electro-oculogram of a 15 sec trial together with the computer print out are given in Fig. 3. The top of the print out represents in decimal numbers, the means and standard deviations of the O K N characteristics analysed. The table is in octal numbers and gives the actual Δp values. The trial starts during a slow movement to the left which is indicated by a down-going signal in the recording and by the first 5 values of the table which end in 3 (table is read from left to right). This is followed by a fast eye movement to the right, indicated by an upward deflection in the recording and by 4 consecutive numbers in the table ending in ϕ (solid underline). Thereafter there is an alternation of series ending in 3 (slow phases to the left) and series ending in ϕ (fast phases to the right) with a few exceptions. For example, there is a single number in the entire table ending in 1 (in brackets) corresponding to a fast phase to the left, which can easily be ident the recording (arrow) and appears ond line of the upper portion

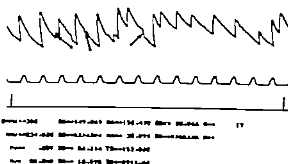


Fig 3. Sample analysis. Upper tracing: electro-oculogram; middle tracing: photoelectric pick-up of luminous stripes; lower tracing: spikes indicating start and end of trial. Stimulus moving from right to left at frequency close to 1 Hz (velocity 45 sec⁻¹ at a center of screen). The upper portion of the print-out appears immediately following the end of the trial. The first number of each line is the code for rapid movements to the right (ϕ) and to the left (1) and for slow movements to the right (2) and left (3). *MMV* mean maximum velocity of fast phase; *MA* mean amplitude; *N* number of nystagmic beats *MV* mean velocity of slow phase *TD* total deviation of the eyes during all slow phases; *SD* standard deviations. Values are in decimal numbers. The lower portion of the print-out represents the table of Δp which can be retrieved if necessary. These are octal numbers from which the first 3 figures are the actual value and the last figure is the code indicating direction and relative velocity as described above. It should be noted that the highest Δp value of each sequence ending in ϕ or 1 is multiplied by 6 prior to print-out of MMV to compensate for the different time base used for fast and slow movements. In this trial, the response is an O.K.N with 17 fast phases to the right which can be identified in the table by solid underlined sequences of octal numbers ending in ϕ. A single fast movement to the left is indicated by the arrow in the E.O G and the bracketed octal number ending in 1. In addition, there are 7 isolated octal numbers ending in 2 (*dotted underline*) representing slow movements to the right, some of hich can be identified in the E.O G (*ringed arrows*)

out. There are also 7 isolated numbers ending in 2 (dashed underline) representing eye movements to the right, which were below the "discriminator" level and therefore were treated as slow eye movements. The first one corresponds to a small amplitude ocular deviation which was probably a relatively "slow" fast phase to the right (left ringed arrow). The second such instance might indicate a brief interruption of a slow phase to the left by a short reversion of the direction (right ringed arrow). In any event, these are minimal sources of error.

Perhaps a more significant error derives from the occasional discarding of terminal segments of slow phases with more than 1 but less than 6 consecutive Δps below the "discriminator" level. In the present example each number in the table ending in 0 or 1 represents 15 msec of eye movement, whereas each ending in 2 or 3 are 90 msec. There are 53 values ending in 0 and 1, therefore 53×15 msec $= 795$ msec which were spend in fast movements. There are 149 values ending in 2 and 3, therefore 149×90 msec $= 13\,410$ msec which were spend in slow phases. The total time spent in both fast and slow phases accounted for by the computer was 14 205 msec. Consequently 795 msec (the difference with 15 sec duration of the trial) were discarded. This represents less than a 6% loss of information concerning the total of the slow phases.

DISCUSSION

The present system allows for the collection and analysis of electro-oculographic data of nystagmus in a manner suitable for the immediate statistical treatment of the phenomenon provided that the samples are taken after the response is fully established. This approach leads to the definition of an ideal beat of nystagmus representative of the population with a mean and standard deviation for each response characteristic. Although the selection of the response elements was arbitrarily made in the present study, the off line portion of the program can be readily enlarged to include other features derived from the data already existing in the table of first derivatives, such as mean duration of the fast and slow phases and mean velocity of the fast phase. It is also possible that acceleration values of eye motion could be obtained by constructing a second table based this time on differences between consecutive Δp values (2nd derivative of eye position). In addition, the various constants involved in the program can be easily changed by altering a single address to adapt it to individual circumstances that may require a different sampling rate, time intervals, ratio between slow and fast phase durations and "discriminator" level. It should be noted that the present system does not provide absolute values of O.K.N. amplitude and velocities. This would require a calibration procedure that utilized at least two eye movements of either known velocity or known amplitude to solve the linear equation which represents the relationship between these response magnitudes and the computer output. This can readily be accomplished in man but requires special training techiques in experimental animals (Wurtz, 1969).

The system described in this communication appears to be ideally suited to both research and clinical applications on the basis of its small size and relatively low cost. The possibility of physical location within the physiologic laboratory allows the undertaking of experiments which involve close-loop type of procedures such as thresholds determinations, where the results of one trial are used to change the stimulus parameters of a subsequent trial (Valciukas, 1972). In the clinical setting the immediate availability of numerical results would allow the rapid quantification of the data and therefore would permit the evaluation of various changes in the testing situation such as the effects of varying the stimulus parameters or exploring the effects of drugs within the same session.

ZUSAMMENFASSUNG

Für die Analyse des Elektrookulogrammes während optokinetischer Nystagmen beim Affen wurde ein Programm für einen kleineren Allzweck-Computer entwickelt. Das Programm sichert sofortige print-outs über die Mittel- und Streuungswerte der maximalen Geschwindigkeit der raschen Phase, die Geschwindigkeit der langsamen Phase, Amplituden, Gesamtabweichung aller langsamen Phasen und über die Zahl der nystagmischen Ausschläge während Versuchsperioden von je 15 Sekunden. Das Programm gewährt die Möglichkeit, auch andere Eigenschaften der Reizantworten auszuwerten, und die Konstanten können den jeweiligen Umständen leicht angepasst werden. Die Zuverlässigkeit des Auswertungsverfahrens konnte in simulierten Leer- und Vollversuchen sichergestellt werden. In Anbetracht der geringen Grösse und der unerheblichen Kosten der Apparatur ist dieses System geradezu ideal für "on-line" Auswertungen und für geschlossene Versuchsanordnungen, bei denen die Ergebnisse der Analyse sogleich wieder in der Reihfolge verwertet werden sollten. Gleicherweise ist die Versuchsanordnung zur klinischen Auswertung normaler und pathologischer Augenbewegungen während derselben Untersuchungsperiode unter den verschiedensten Bedingungen geeignet.

RÉSUMÉ

Un programme pour un petit ordinateur d'usage général a été developpé afin d'analyser des électrooculogrammes de nystagmus optocinétique chez les singes. Ceci permet d'obtenir un « print-out » immédiat des moyens et des deviations standard de la vitesse maximum des phases rapides, vitesse des phases lentes, amplitude, déviation totale pendant les phases lentes, et le nombre des battements nystagmiques prenant place durant des essais de 15 secondes. Il permet également de mesurer d'autres aspects de réponse et les constantes peuvent être aisément modifiées pour application à des circonstances individuelles. La validité de ce système a été verifiée autant par la stimulation que par l'analyse des consignes. La taille réduite et le cout relativement bas de l'appareil rend ce système idéal pour les experiences « on—line » sur boucles fermées pour le laboratoire de physiologie et pour la quantification rapide des renseignements d'ordre clinique qui permettraient l'évaluation de mouvements oculaires normaux et anormaux sous une variété de conditions pendant la même séance.

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Acta Otolaryng 76 450–454 1973

SERUM OSMOLALITY IN PATIENTS WITH MENIERE'S DISEASE

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(Received March 30, 1973)

Abstract Hyperosmolality in serum was found in 19 of 56 patients with Menière s disease. The cause of the moderately elevated serum osmolality has not yet been identified The serum sodium and potassium concentrations were within normal limits. The hyper osmolality may indicate that Menière's disease is a disorder afflicting the entire organism. Hyperosmolality alternating with normal osmolality was seen in several patients, a phenomenon which may be in accordance with the general fluctuating character of the disease The elevation of serum osmolality after the peroral consumption of glycerin in patients with Menière s disease was studied and related to the hearing loss, and no correlation was found.

Spontaneous variations in both hearing (Lindsay 1949 1960 Meurman & Grahne, 1956 Opheim & Flottorp 1957 Schuknecht 963 Goodman, 1965 Enander & Stahle,) and caloric response (Angelborg et al., 1) occur in patients with Menière s disease. eduction of the hearing loss, comparable with the spontaneous remissions, may be promptly induced with glycerin to an extent that depends on the phase of the disease (Klockhoff & Lindblom, 1966 1967 *a*) In practice glycerin administered perorally in doses of 1 2 g kg body weight results in a rapid though transient improvement of hearing in roughly every second test. The effect of glycerin on the vestibular function is more complex. The caloric response may be increased or reduced or may remain unchanged, regardless of the effect on hearing Positional nystagmus of the direction-changing type has been a common finding after glycerin administration in normal controls, as well as in patients with Menière s disease (Stahle et al., 1970 Angelborg et al., 1971).

Glycerin is a polyhydric alcohol which raises the osmolality of the blood serum. This hyperosmolality is thought to reduce the intralabyrinthine hydrops, resulting in improvement in hearing presumably due to reduction of the hydrodynamic damping of th receptor organ (Klockhoff & Lindblom, 1966 Angelborg et al. 1971). Administration of glycerin changes the osmotic pressures of th inner-ear fluids, as has been shown in rats by Bosher & Warren (1971) Some of our patients report temporary improvement in hearing with less distortion of sound after the ingestion of ethyl alcohol, which has been regarded as being the most common cause of an elevated serum osmolality (Robinson & Loeb 1971).

Under normal conditions, the serum osmolality is primarily determined by the concentration of inorganic ions, principally sodium and potassium and hyperosmolality is a very unusual phenomenon. Under certain pathological conditions, such as in diabetes mellitus and uremia plasma glucose and urea respectively are capable of causing hyperosmolality

The resemblance between the spontaneous and the glycerin-induced variations in hearing caused us to focus our attention on serum osmolality The purpose of the present work was (1) to investigate the serum osmolality of

This study has been supported by a grant from the Paul Frenckner Fund and by the Swedish Medical Research Council (Project No B73-17X 1905-01)

patients with Menière's disease, and (2) to study the effect of glycerin consumption on serum osmolality and hearing.

METHOD AND MATERIAL

The freezing-point-depression method, using a Knauer osmometer was used for determination of the serum osmolality The subjects were tested early in the morning with empty stomachs. The patients belonging to group B (see below) received a single dose of glycerin perorally (1.2 g/kg body weight, diluted with the same amount of saline and flavoured with lemon juice) Two hours after the glycerin administration, when the maximal blood concentration was to be expected (Stahle et al., 1970), a fresh blood sample was taken for a second measurement of osmolality Forty-three of the 56 patients with Menière's disease were tested in this way

The material consisted of three groups, the *control group* (healthy individuals), the *patient group A* (patients with common ENT disorders) and the *patient group B* (patients with Menière's disease)

The *control group* comprised 25 young and healthy individuals belonging to the standard *test group of the hospital laboratory* The serum osmolality in this group ranged between 281 and 297 mOsm/kg (2×S.D) which is looked upon as the normal range for osmolality

Patient group A consisted of 50 patients admitted to our ENT clinic for common types of surgical procedure: rhinoplasty tonsillectomy tympanoplasty and stapes surgery No patient had a history of vertigo.

Patient group B consisted of 56 patients who had had a verified Menière's disease for a considerable time.

RESULT

The findings with respect to serum osmolality for the two groups of patients are presented in Table I and Fig. 1 Figures exceeding 297 mOsm/kg are regarded as abnormal.

Table I. *Serum osmolality in patients with Menière's disease and in controls*

	Normal osmolality		Hyper osmolality		Total no. of patients
	n	%	n	%	
Patient group A (without Menière's disease)	45	90	5	10	50
Patient group B (with Menière's disease)	37	66	19	34	56

The presence of hyperosmolality among patients with Menière's disease is greater than that among the patients without Menière's disease. The difference is significant at the 1% level (χ^2 test).

In patient group B (cases of Menière's disease) the mean value of the serum osmolality was 295.1 mOsm/kg (S.D 11.5). In patient group A, the corresponding figures were 286.4 mOsm/kg (S.D 9.0).

Sodium and potassium were measured in the same blood samples, as had been submitted for osmolality determination. The results have been grouped in Table II. The normal ranges presented are those of our hospital laboratory

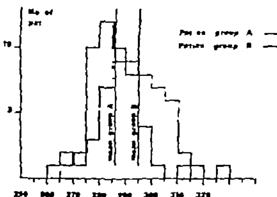


Fig. 1 Distribution of the values for in patient group A (cases without and group B (cases with Meni

As a result of the hyperosmolality in group B cases, we expected to find an elevated serum-sodium value but as will be seen in the table this was not the case. Even if the individuals with hyperosmolality are considered separately the serum sodium and potassium levels are not raised, with one exception. Therefore the hyperosmolality frequently noted in our group B cases cannot be explained by elevated sodium or potassium concentrations.

Serum osmolality after glycerin consumption

Serum osmolality was measured both before and 2 hours after glycerin administration in 43 of the 56 patients with Menière s disease. The elevation ranged between 11 and 30 mOsm kg in 35 out of 43 patients (Fig. 2)

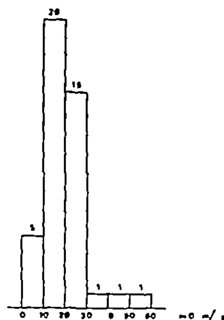


Fig The elevation of serum osmolality after glycerin administration in 43 patients with Menière disease The figures above the piles indicate the number of patients.

Serum osmolality and hearing loss after glycerin administration

As was mentioned in the introduction glycerin may induce a transient hearing improvement. In the light of this fact, we have compared the serum osmolality in the 56 patients with Menière s disease with the results of the glycerin tests (Table III), which were judged to be either positive or negative according to the following criteria. A positive glycerin test refers to an improvement of the pure tone threshold of at least 10 dB in the three adjacent octave bands with a simultaneous improvement of the speech-discrimination score exceeding 12% 2 hours after glycerin administration There was no correlation between the effects of glycerin on hearing and serum osmolality nor did the 19 individuals with initial hyperosmolality (cf Table I) react differently after the ingestion of glycerin, compared with the remaining 37 patients with Menière s disease

Table II *Serum sodium and potassium concentration*

	Serum sodium, mean value	Serum potassium, mean value
Patient group A	138 8 mEq l (S D 1)	4 3 mEq l (S D 0 3)
Patient group B	139 9 mEq l (S D 3 5)	4 0 mEq l (S D 0 4)
Normal range	134 146 mEq l (S D 3)	3 6-4 6 mEq l (S D 0 5)

Glycerin-test result and elevation of serum osmolality

The elevation of serum osmolality resulting from the standard dose of glycerin was compared with the effect of glycerin on hearing in 43 of our patients with Menière s disease A correlation between the increase of osmolality and the hearing gain might have been expected However no definite correlation was found. The same was also true of those patients who initially showed serum hyperosmolality (Table IV).

Spontaneous fluctuations in serum osmolality

Repeated measurements of serum osmolality at intervals of several months were perform

Table III. *Comparison between serum osmolality and the results of glycerin tests*

	Normal serum osmolality	Hyper osmolality	Total
Glycerin test positive	15	8	23
Glycerin test negative	22	11	33
Total no. of patients	37	19	56

in 16 of the patients with Menière's disease. Hyperosmolality alternating with normal osmolality was seen in 7 patients. This phenomenon may be in accordance with the general fluctuating character of the disease which is particularly distinctive with regard to hearing. However the relationship between spontaneous changes of serum osmolality and fluctuations in cochleovestibular symptoms has not yet been studied.

DISCUSSION

The cause of the serum hyperosmolality in Menière's disease reported here has not been identified. Elevation of serum osmolality can be found in patients with renal diseases, burn injuries and some brain disorders. Diabetes mellitus and uremia are two common causes. Marked fluid loss, as well as an increase of sodium potassium, urea and glucose may elevate the serum osmolality The ingestion of alcohol is looked upon as the commonest cause.

Table IV *Elevation of serum osmolality compared with the effects on hearing 2 hours after glycerin administration*

	Elevation < 20 mOsm/kg	Elevation > 20 mOsm/kg	Total
Glycerin test positive	11	7	18
Glycerin test negative	14	11	25
Total no. of patients	25	18	43

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The hyperosmolality found in patients with Menière's disease cannot be explained by any of the causes mentioned above The serum sodium and potassium concentrations were within normal limits. None of our patients had diabetes mellitus and the urinary-glucose tests were negative and no proteinuria was found. The serum-urea concentration was measured in five patients and was found to be normal.

Some of our patients with Menière's disease had been periodically treated with diuretics. *Hygroton*® (chlortalidon) had most frequently been used (Norell & Stahle 1962 Klockhoff & Lindblom 1967 *b* 1968). Hyperosmolality resulting from such medication has not been reported and diuretics would rather be expected to lower the osmolality due to the increase in sodium excretion.

The serum hyperosmolality observed in one-third of our patients with Menière's disease may be regarded as manifesting a general disturbance of homeostasis, although the clinical symptoms mainly originate from the cochleovestibular pathways. Hyperosmolality in the endolymphatic space, causing hydrops, may occur simultaneously This would be in line with the theories recently presented by Godlowski (1972), who suggested that the inner ear disturbance in Menière's disease may be an expression of an immunometabolic disorder similar to diabetic retinopathy He indicated that endolymphatic hydrops is an extension of a pathological process involving the whole organism, predominantly concentrated in the inner ear He suggested that the primary cause of the endolymphatic hydrops may be a reduction of the depolymerizing action of hyaluronidase, caused by various etiological factors (hereditary infective, toxic, etc.). This was thought to cause an accumulation of hydrophilic hyaluronate mucopolysaccharides in various parts of the organism and, within the endolymphatic space to result in hydrops of varying degrees.

As a result of the hyperosmolality in group B cases, we expected to find an elevated serum-sodium value, but, as will be seen in the table this was not the case. Even if the individuals with hyperosmolality are considered separately the serum sodium and potassium levels are not raised, with one exception. Therefore the hyperosmolality frequently noted in our group B cases cannot be explained by elevated sodium or potassium concentrations.

Serum osmolality after glycerin consumption

Serum osmolality was measured both before and 2 hours after glycerin administration in 43 of the 56 patients with Menière s disease. The elevation ranged between 11 and 30 mOsm/kg in 35 out of 43 patients (Fig. 2).

Serum osmolality and hearing loss after glycerin administration

As was mentioned in the introduction, glycerin may induce a transient hearing improvement. In the light of this fact, we have compared the serum osmolality in the 56 patients with Menière s disease with the results of the glycerin tests (Table III), which were judged to be either positive or negative according to the following criteria. A positive glycerin test refers to an improvement of the pure tone threshold of at least 10 dB in the three adjacent octave bands with a simultaneous improvement of the speech-discrimination score exceeding 12% 2 hours after glycerin administration. There was no correlation between the effects of glycerin on hearing and serum osmolality nor did the 19 individuals with initial hyperosmolality (cf Table I) react differently after the ingestion of glycerin, compared with the remaining 37 patients with Menière s disease.

Table II *Serum sodium and potassium concentration*

	Serum sodium, mean value	Serum potassium, mean value
Patient group A	138.8 mEq/l (S D 2 3)	4.3 mEq/l (S.D 0.3)
Patient group B	138.9 mEq/l (S.D 3.5)	4 0 mEq/l (S D 0 4)
Normal range	134–146 mEq/l (S.D 3)	3 6–4 6 mEq/l (S.D 0.25)

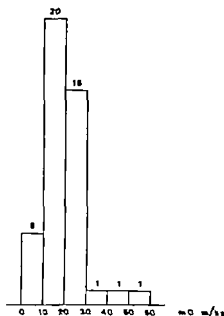


Fig 2. The elevation of serum osmolality after glycerin administration in 43 patients with Menière's disease. The figures above the piles indicate the number of patients.

Glycerin-test result and elevation of serum osmolality

The elevation of serum osmolality resultin from the standard dose of glycerin was con pared with the effect of glycerin on hearin in 43 of our patients with Menière s diseas A correlation between the increase of osmo lality and the hearing gain might have bee expected. However no definite correlatio was found. The same was also true of thos patients who initially showed serum hypero molality (Table IV)

Spontaneous fluctuations in serum osmolality

Repeated measurements of serum osmolali at intervals of several months were perform

There is also a remote possibility that serum hyperosmolality may be a reflexogenic phenomenon which is secondary to the inner-ear disorder The serum osmolality in that case is raised in order to increase the withdrawal of water from the endolymph and diminish the hydrops.

Determination of the serum osmolality on a single occasion has shown hyperosmolality in 19 out of 56 patients (34%). Measurement on two occasions at intervals of several months has revealed hyperosmolality in 7 out of 16 patients (44%) Provided that the variations in osmolality can be interpreted as a cyclic phenomenon, the frequency of hyperosmolality could be expected to be even higher if the number of examinations was further raised. It should be stressed that the elevation of the serum osmolality may be an inconstant phenomenon analogous with other fluctuating symptoms.

ZUSAMMENFASSUNG

Hyperosmolalität im Serum ist bei einem Drittel von 56 Patienten mit Menièrescher Krankheit angetroffen worden. Die Ursache der mässig erhöhten Serumosmolalität ist noch nicht klargelegt worden. Natrium- und Kaliumgehalt im Serum waren normal. Hyperosmolalität kann ein Zeichen dafür sein, dass die Menièresche Krankheit eine den ganzen Organismus angehende Krankheit ist. Ein Wechsel zwischen Hyperosmolalität und normaler Osmolalität ist bei mehreren Patienten angetroffen worden. Dies kann als Ausdruck für den fluktuierenden Verlauf der Krankheit aufgefasst werden. Die Steigerung der Serumosmolalität nach peroraler Glyzerindosis bei Patienten mit Menièrescher Krankheit ist studiert und mit der Herabsetzung des Gehöres verglichen worden.

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